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# LOW-LEVEL SARIN (GB) VAPOR EXPOSURE IN THE GOTTINGEN MINIPIG: EFFECT OF EXPOSURE CONCENTRATION AND DURATION ON PUPIL SIZE

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**14. ABSTRACT**
One of the goals of this study was to determine the lowest sarin (GB) vapor concentration of physiological significance. During the whole body exposure to GB vapor of a living species, the first noticeable effect is constriction of the pupil (miosis). The Gottingen minipig was chosen as a model for studying the effects of GB vapor because of the anatomical and physiological similarities between humans and minipigs. The minipigs were secured in a sling during exposure and their pupil size was continuously monitored under dim-light conditions using an infrared light-sensitive video camera. High-resolution images of the eye were collected before, during, and after each exposure. Pupil area was then quantified using a custom-designed software package. Ordinal regression was used to fit various response models to the data. The effective concentrations resulting in miosis in 50% ($EC_{50}$) of the exposed subjects were determined for 3 exposure-durations (10, 60, and 180 min). The median effective dose ($ECT_{50}$) associated with miosis was not constant over time. The value of the toxic load exponent was essentially independent of the model used: $1.32 \pm 0.18$ (95% confidence interval of 1.14 to 1.50). Since this interval did not overlap one, Haber's rule was found to be an inappropriate time dependence model for this dataset.

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Toxic load model, Probit slope, Inhalation, Cyclosarin, Gottingen, Minipig, Swine, Low level, Concentration, Duration, $ECT_{50}$, Pupil, Inhalation, Miosis, GF, Ct

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## PREFACE

The work described in this report was authorized under Project No. 201400, Low-level Toxicology. The work was started in March 2004 and completed in September 2004. The experimental data are contained in Laboratory Notebook No. 04-0002 and on compact discs. Raw data and the final report from this study are stored in the Toxicology Archives, E-3150, Aberdeen Proving Ground, MD 21010-5424.

While conducting this study, investigators adhered to the "Guide for the Care and Use of Laboratory Animals," National Institutes of Health Publication No. 86-23, 1985, as promulgated by the committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, Washington, DC. These investigations were also performed in accordance with the requirements of AR 70-18, "Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs," and the U.S. Army Edgewood Chemical and Biological Center (ECBC) Institutional Animal Care and Use Committee, which oversees the use of laboratory animals. This project's assigned IACUC protocol No. 02-341 was approved on 6 August 2002.

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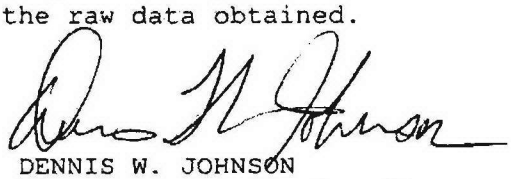
## QUALITY ASSURANCE

This study, conducted as described in Protocol 02-341, was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792 (effective 17 Aug 1989). The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

| Phase Inspected | Date | Reported |
|---|---|---|
| Study parameters and exposure | 26 Sep 02 | 26 Sep 02 |
| | 06 Feb 03 | 06 Feb 03 |
| Data and Final Report | 28 Jul 04 | 28 Jul 04 |

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.



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# LOW-LEVEL SARIN (GB) VAPOR EXPOSURE IN THE GOTTINGEN MINIPIG: EFFECT OF EXPOSURE CONCENTRATION AND DURATION ON PUPIL SIZE

## 1. INTRODUCTION

The primary objective of the current research study is to thoroughly understand the dose/response relationship of traditional chemical warfare (CW) nerve agents. Constriction of the pupil (miosis) is often the first noticeable effect of exposure to vapor on humans, thereby making it an ideal biological endpoint for determining and modeling threshold dose/response relationships. Pupil constriction is thought to be a local effect caused by direct contact between a nerve agent and the eye. High levels of cholinesterase (ChE) activity have been documented in the extraocular muscles, retina, eyeball retractor muscles, and iris.[1] Nerve agent vapors can rapidly transverse the conjunctiva and inhibit local ChE, resulting in the stimulation of muscarinic receptors at the sphincter muscles of the iris and the ciliary muscle of the lens. This stimulation can cause pupil constriction and problems with accommodation. The inability of the pupil to dilate can also result in the loss of dark adaptation.[2] Given that military operations are often conducted at night, threshold levels for nerve agent intoxication in dim-light situations need to be determined.

In order to gauge the biological impact of nerve agent vapor exposure on the eye, the probability of eye responses to appropriate exposure parameters have to be quantitatively related. Traditionally, inhalation and ocular toxicology has used dosage (expressed by the product of exposure concentration (C) and exposure-duration (T)) as a metric of toxicant exposure.[3] The range of dosages associated with physiological effects is best described by a normal distribution of log (effective dosages). Toxicologists commonly characterized this distribution by using two parameters: the median effective dose, $ECT_{50}$ (the dosage at which 50% of the exposed individuals will exhibit a specified biological response), and the probit slope, $m$ (which equals the inverse of the standard deviation, $\sigma$). Though the normal distribution is continuous, quantal data (response versus no response) are used to estimate the parameters ($ECT_{50}$ and $m$) of effective dosage distribution via maximum likelihood estimation (MLE).[4]

Historically, the time dependence of CW agent toxicity has been modeled using Haber's Law, which assumes that $ECT_{50}$ is constant with respect to the value of exposure-duration.[5] However, this concept has been found to be inadequate for assessing biological effects from exposure to many acutely toxic gases and aerosols.[6] Recent efforts have resulted in data with low vapor concentration exposures over long periods, which can best be described with a toxic-load model.[6,7] In the toxic-load model, dosage is not used to quantify the amount of toxic material received. Instead, a new term, toxic load (TL), has been developed and extensively used, with TL equaling $C^nT$ being a typical form.[8] The TL exponent, n, is toxicant and exposure scenario dependent. For the TL model, the median effective TL ($ETL_{50}$) is assumed to equal a constant. The median effective dosage ($ECT_{50}$) no longer remains constant but is dependent on T. The differences between the models are illustrated in Figure 1.

Previously, Mioduszewski et al. found that instead of Haber's Law, the TL model better approximated the occurrence of miosis from GB vapor exposure in rats.[7] However, rodents

are known to have a greater resistance than other mammals to poisoning from CW nerve agents due to the organophosphate scavenging properties of carboxylesterases in their plasma and organs.[9-12] Thus, to develop a human miosis model, additional data from a non-rodent species are needed. Pigs are in many ways similar in anatomy and physiology to humans.[13] This study estimates effective (miosis) concentrations of the nerve agent, sarin (GB), as a function of exposure-duration in the Gottingen minipig and determines the dependency of the median effective dosage ($ECT_{50}$) over time.

## 2. MATERIALS AND METHODS

### 2.1 Gottingen Minipigs.

Forty (20 of each gender) sexually mature male (3 to 4 months old) and female (4 to 5 months old) Gottingen minipigs (Sus scrofa) were obtained from Marshall BioResources USA (North Rose, NY). The pigs were shipped to the testing facility in batches of 10. Each batch contained pigs of a single gender. Testing of each batch was completed before the arrival of the next batch; male and female pigs were never present at the facility concurrently.

Upon arrival at the testing facility, the minipigs underwent an initial health examination by the attending veterinary staff. The pigs were then quarantined for a minimum of 3 days, after which time, the research personnel familiarized the pigs to testing procedures such as constant handling by personnel, location changes within the facility, and adaptation to a sling apparatus. While the pigs were in their cages, they were given unfettered access to play toys (hanging chains, bunny balls) and food treats.

### 2.2 Surgical Procedure.

Silicone catheters (Bard access systems, 6.6 or 9.6 Fr.) were implanted in the minipigs to facilitate the draw of blood samples. Each surgical site on a minipig (lateral neck from mandible to shoulder and mid dorsally between the shoulder blades) was prepared for aseptic surgery by close-clipping the area and applying a surgical scrub, chlorhexidine, followed by an application of isopropyl alcohol. The area was then covered with sterile drapes and the minipig was positioned for surgery on a heated surgical table.

Throughout the surgical procedure, an Electrocardiogram, a temperature probe, a pulse oximeter, and a respirator were used to monitor the minipig. A catheter, impregnated with heparin and antimicrobial agent, was implanted in an external jugular vein of the minipig and advanced to the level of the anterior vena cava or right atrium. A subcutaneous tunnel, extending from the surgical site (adjacent the jugular vein) to the exit site in the dorsal midline, was created with a hollow stainless-steel rod. The catheter was filled with sterile heparin saline (1/100), grasped, and pulled through the dorsum to the ventral neck incision with at least 6 in. exposed above the surgical site. The position of the catheter was adjusted so that blood samples could be readily obtained. The catheter was secured by tying sutures (minimum 2) around the vein. A catheter loop leading from the vein was also secured to the subcutaneous tissues using sutures. Once the catheter was appropriately adjusted, it was secured at the dorsal exit site and the

incisions were closed. The catheter was locked with 1/100 sterile heparin saline. Triple antibiotic ointment was placed on both incisions.

The minipigs were given at least 3 days to recover from the surgery. To minimize the risk of infection, the vascular access port of each indwelling catheter was flushed with heparinized saline and each minipig was given analgesics (buprenorphine 0.01 - 0.05mg/kg, BD) for at least 24 hr, postoperatively.

2.3 Blood Sample Collection.

After the 3-day recovery period, the minipigs were exposed to the GB nerve agent vapor. During the agent exposures, the indwelling catheter was maintained by a continuous intravenous infusion of lactated Ringers solution. Blood samples to assess cholinesterase inhibition[14] and internal agent levels via GB regeneration assays[15] were taken from the minipigs prior to the start of an exposure and at periodic intervals throughout. The samples were taken approximately every 2 min during the 10-min exposure, every 15 min during the 60-min exposure and every 20 min during the 180-min exposure. The total volume of blood drawn did not exceed 1% of the body weight of a minipig over a 1-week span. An equivalent volume of Lactated Ringers replaced drawn sample volumes.

2.4 Inhalation Chamber.

Whole-body exposures of the minipigs to the GB nerve agent vapor were conducted in a 1000-liter dynamic airflow inhalation chamber. The 6-sided, Rochester style chamber was constructed of stainless steel with Plexiglas windows on each side. The chamber interior was maintained under negative pressure (0.50" $H_2O$), which was monitored with a calibrated magnehelix (Dwyer, Michigan City, IN). A thermoanemometer (Model 8565, Alnor, Skokie, IL) was used to monitor airflow at the chamber outlet.

The chamber GB vapor concentration was monitored and analyzed by 2 sampling methods. The first method was a quantitative technique using solid sorbent tubes (Tenax/Haysep) to trap the GB vapor. The GB was then thermally desorbed and a GC analysis performed (HP Model 6890, Agilent Technology, Baltimore, MD). The second method was a continuous monitoring technique using a phosphorus monitor (HYFED, Model PA260 or PH262, Columbia Scientific, Austin, Texas). Output from the HYFED provided a continuous strip chart record of the rise, equilibrium, and decay of the chamber vapor concentration during an exposure.

After the chamber attained an equilibrium of $t_{99}$ (99% of the target concentration for the run), solid sorbent tube samples were drawn every 10 min from the middle of the chamber. Each sample draw lasted 1 to 5 min depending upon chamber concentration and exposure-duration. In general, lower GB concentrations required longer sample draw times (5 min) and higher GB concentrations required shorter sample draw times (1 min). All sample flow rates for the solid sorbent tube systems were controlled with calibrated mass flow controllers (Matheson Gas Products, Montgomeryville, PA). The flow rates were verified before and after sampling by temporarily connecting a calibrated flow meter (DryCal®, Bios International,

Pompton Plains, NJ) in-line to the sample stream. The HYFED was used to monitor an entire run. Physical parameters (chamber airflow, chamber room temperature, and relative humidity) were monitored during the exposure and recorded approximately every 10 min.

2.5 Solid Sorbent Tube System.

The automated solid sorbent tube sampling system consisted of 4 parts:

(1) A heated sample transfer line
(2) A heated external switching valve
(3) A thermal desorption unit
(4) A gas chromatograph.

A stainless steel sample line (1/16" o.d. x 0.004" i.d. x 6' length) extended from the middle of the chamber to an external sample valve. The sample line was commercially treated with a silica coating (Silicasteel® Restek, Bellefonte, PA) and covered with a heated (60 °C) sample transfer line (CMS, Birmingham, AL). Nerve agent absorption onto sample surfaces was minimized by the combination of the coating and the 60 °C temperature of the transfer line.

From the transfer line, the sample entered a heated (125 °C), 6-port gas-switching valve (UWP, Valco Instruments, Houston, TX). In the by-pass mode, vapor from the chamber continuously purged through the sample line and out to a charcoal filter. In the sample mode, the gas sample valve redirected nerve agent vapors from the sample line to a Tenax TA/Haysep sorbent tube (60 to 80 mesh) located in the thermal desorption unit (ACEM-900, Dynatherm Analytical Instruments, Kelton, PA). Temperature and flow programming within the Dynatherm desorbed nerve agents from the sorbent tube directly onto the GC column (RTX-5, 30-m length, 0.32-mm i.d., 1-mm thickness), which resulted in flame photometric detection (FPD - phosphorus mode).

The solid sorbent tube sampling system was calibrated by the direct injection of external standards (GB µg/ml) into the heated sample line of the Dynatherm. This way, injected nerve agent standards were subjected to the same sampling and analysis stream as the chamber samples. A linear regression fit ($r^2 = 0.999$) of the standard data was used to compute the GB concentration of each chamber sample.

2.6 Chemicals.

Isopropyl methyl phosphonofluoridate (Sarin or GB) was used for all the vapor exposures conducted in this study. Chemical agent standard analytical reagent material (CASARM)-grade GB (lot # GB-U-6814-CTF-N (GB2035) was verified (usually 98.3 + 0.48 wt.% pure as determined by quantitative $^{31}$P-NMR) and stored in sealed ampoules containing nitrogen. The ampoule contents were used either as neat agent for vapor generation or as the basis for the daily preparation of external standards. Triethylphosphate (99.9% purity), obtained from Aldrich Chemicals, Milwaukee, WI, was used as the internal standard for the GB purity assays. Analysis for agent impurities was conducted using acid-base titration, Gas

Chromatography/Mass spectrometry (GC-MS), and $^1$H NMR. Based on mole ratios, acid-base titration has been proven to show the impurity percentages listed in Table 1. Testing conducted with GC-MS positively identified DIMP, Diisopropyl phosphonofluoridate, Tributylamine, and Isopropyl ethylphosphonofluoridate, but did not quantify the amounts. Tributylamine, with a concentration of < 0.1 wt% of GB, was also confirmed through $^1$H NMR testing.

2.7 Vapor Generation.

Saturated GB vapor streams were generated by flowing nitrogen carrier gas through a glass vessel (multi-pass saturator cell) that contained liquid GB. The saturator cell consisted of a 100-mm long, 25-mm o.d. cylindrical glass tube with two (inlet, outlet) vertical 7-mm o.d. tubes connected at each end. The main body of the saturator cell contained a hollow ceramic cylinder that functions to increase the contact area between the liquid nerve agent and the nitrogen. The nitrogen was passed 3 times along the surface of the wetted ceramic cylinder before it was allowed to exit through the outlet arm of the glass cell. The saturator cell body was immersed in a constant temperature bath so that a combination of nitrogen flow and temperature could regulate the amount of nerve agent vapor going into the inhalation chamber.

The entire apparatus was contained within a generator box mounted atop the inhalation chamber. Typically, the saturator cell was loaded with 2 to 4 ml of liquid nerve agent (CASARM grade). To maintain the integrity of the liquid nerve agent within the cell, a continuous low flow rate (1 to 2 ml/min) of the nitrogen was used. This setup was capable of precisely generating GB vapor over a concentration range of 0.001 to 2.0 mg/m$^3$.

2.8 Sling Apparatus.

A sling was used to restrain each minipig during the exposure to the GB nerve agent vapor. The frame of the sling was constructed of airtight stainless steel pipe and Swagelok™ fittings. The slings were custom designed (Lomir Biomedical, Inc., Malone, NY or Canvas and Awning supplies, White Marsh, MD) to fit the build and size of the minipigs. The body of each sling was made of canvas, which contained 4-leg holes so that it could be adjusted comfortably around a pig. The sling also had two 2 straps that secured over the shoulders and hips. A muzzle harness was placed over the snout and secured both laterally and ventrally to the stainless-steel framing in order to prevent a pig from moving its head from side-to-side. A minipig was held in place by the sling apparatus so that a consistent angle and distance (40 in.) from the infrared (IR) camera to the eye of the pig was maintained. The harness was fitted so that it did not interfere with a pig's ability to breathe.

2.9 Infrared Camera.

A Sony CCD black and white video camera (model XC-ST50), equipped with 2-IR 100-candlepower spotlights, was focused on the left pupil of a minipig for the duration of the nerve agent exposure. The distance between the camera and the eye was standardized at approximately 40 in. and the images were shot through the external Plexiglas of the exposure chamber at a consistent angle. Plexiglas does not interfere with the quality of IR images.

Sequential images of the eye, under dim light conditions, were digitally captured for the analysis and calculation of the pupil area at a later time. The GB exposures were for 10, 60, or 180 min. However, the minipigs were required to remain in the exposure chambers for an additional 15 min for out-gassing. The pigs were then removed from the chambers and additional images were captured for 50 to 60 min to ensure no further decrease in pupil area.

2.10 Infrared Pupillometry.

The basis of IR pupillometry is the entry of light through the pupil, reflection off the retina and back through the pupil to the camera lens, producing the image of a bright pupil. The IR method causes no constriction of the pupil and allows pupil area measurements to be calculated under dim light conditions. The IR method maximizes the pupil area and provides for measurements in a realistic environment.

Real-time images of the pupils during exposure were captured and saved for quantifying pupil area off-line (Figure 2). The images were captured, filtered and quantified using a custom designed software program. During the exposures, the computer program displayed a live video from the camera and allowed the operator to capture and display images as often as needed; the operator also selected and saved certain images for later analysis. The images were classified according to light intensity on a scale of 256 different densities of gray (0 = Black, 255 = White). The computer program quickly identified any single density or range of densities (densities bandwidth) that allowed the separation of bright and dim objects in an image.

The operator was able to measure and repeat analysis of an image until a true representation was obtained. The operator was also able to select a density bandwidth and a pixel in the area to be measured. The program then drew a border around all the pixels within the gray scale density bandwidth of the selected pixel. For example, if the operator specified a density bandwidth of plus or minus 12 and had selected a pixel that had a density value of 115 the computer would select pixels with a density value ranging from 103 to 127 outward from the selected pixel and draw a border around that section (Figure 3). The operator could adjust the density bandwidth and the selected pixel until a representative border for the object to be measured had been obtained.

Measurements (such as length, height, and center) were calculated by determining the video image coordinates of every point on the border of the image. The points with the highest and lowest X- and Y-axis values (far left, far right, top, and bottom coordinates on the border) were used to locate the center and determine radii. Y values increase down the Y-axis because a video screen updates its image from top left to bottom right. In eq 1, (x,y) represent the coordinates of the points along the X and Y axes of a graph.

$$\text{Center } (x,y) = \frac{\text{Far Left } (x,y) + \text{Far Right } (x,y)}{} \quad (1)$$

Horizontal Radius = Center (x) – Left (x)

Top Radius = Center (y) – Top (y)
Bottom Radius = Bottom (y) – Center (y)

Elliptical Area = Horizontal Radius x (Top or Bottom) Radius x Pi

Equation 1 enables the calculation of the whole geometric area based on the measurements of only the visible portion of a pupil and allows the quantification of the area of pupils partially obstructed by an eyelid as shown in Figure 4.

The coordinates for the pupil in Figure 4 are listed below:

Far Left (17, 50)
Far Right (107, 42)
Top (66, 25)
Bottom (56, 69)

The calculation for the center coordinates is given as

$$\text{Center } (x,y) = \frac{(17,50) + (107,42)}{2} = (62,46).$$

The calculation for the Horizontal Radius is given as

Horizontal Radius = 62 – 17 = 45.

The calculation for the Bottom Radius is given as

Bottom Radius = 69 – 46 = 23.

Therefore, the Elliptical Area is calculated as

Elliptical Area = 45 x 23 x 3.1416 = 3251.556.

A minipig was classified as testing "positive" for miosis if pupil area were reduced by 50% at any time during either the GB exposure or the post-exposure observation period.

2.11 Design and Data Analysis.

To determine the progression of experimental exposure concentrations, the up-down method[16] was used with an assumed probit slope of 10. The binary response used for executing this method was the presence or absence of miosis. For this study, miosis has been

defined as the post-exposure pupil area being 50% or less of the baseline pupil area. The method of maximum likelihood estimation (MLE)[17] was used on the resulting quantal data to calculate $ECT_{50}$ (miosis) values (and associated asymptotic 95% confidence intervals) for each of the 6-gender exposure-duration groups. The MLE calculations were also performed on a pupil diameter basis. An example of an MLE calculation is presented in Appendix A.

Ordinarily the use of the up-down method does not provide meaningful information on the slope of the dose/response curve as a function of vapor concentration (also known as the probit slope). Not enough subjects are normally tested (usual no. tested is between 6 and 10) in an up-down experiment to permit reliable estimation of the variance of the distribution of responses. However, data from several up-down experiments can be combined together to form a subject pool large enough (between 30 to 40 subjects) for obtaining a variance estimate. The resulting dataset can then be analyzed via traditional probit analysis[4] or ordinal logistic regression in order to obtain a probit slope estimate.[8,18,19]

For the purpose of modeling the response distribution, either eq 2 or eq 3 was used:

$$Y_N = (Y_P - 5) = k_0 + k_C(\log_{10} C) + k_T(\log_{10} T) + k_S(Sex) + k_{TS}(\log_{10} T)(Sex) \qquad (2)$$

$$Y_N = (Y_P - 5) = k_0 + k_C(\log_{10} C) + \sum_i^2 k_{Time,i}(Time)_i + k_G(Gender) + \sum_i^2 k_{TimeG,i}(Time \times Gender)_i \qquad (3)$$

where $Y_N$ is a normit; $Y_P$ is a probit; the k's are fitted coefficients; C is vapor concentration; and T and Time are the exposure-durations. For gender differences, Sex is a covariate that equals 1 for males and -1 for females, and Gender is a 2-level factor (equaling 1 if male or 0 if female). $Y_N$ equals –1, 0 and 1 at the 16-, 50-, and 84-% response levels. In eq 2, the exposure-duration is treated as a covariate, and in eq 3, Time (T) is treated as a 3-level factor (using indicator variables) to account for the effects of 3-exposure-durations of 10, 60 and 180 min. The constants $k_C$ and $k_T$ in eq 2 are the probit slopes for concentration and time, respectively. For ordinal regression using eqs 2 and 3, $k_0$ is adjusted for different quantal response levels. In eq 3, $k_0$ represents the fit for Time equal to 10 min with female minipigs. Adding the term, $k_G$ Gender, gives the fit for an exposure of 10 min with male minipigs. The indicator variables for duration in eq 3, $(Time)_i$, and $(Time \times Gender)_i$, are defined as follows:

a. $(Time)_1$ equals 1 for a 60-min duration and 0 otherwise; and $(Time)_2$ equals 1 for an 180-min duration and 0 otherwise.

b. $(Time \times Gender)_1$ equal 1 for a 60-min duration with male minipigs and 0 otherwise; and $(Time \times Gender)_2$ equal 1 for a 180-min duration with male minipigs and 0 otherwise.

The ratio ($k_C$ / $k_T$) equals the toxic load exponent, $n$. If this ratio is not different (with statistical significance) from one, then Haber's Law is appropriate for modeling the toxicity. Otherwise, the classic toxic load model ($C^nT$) is the proper approach, assuming that there is no significant curvature in the experimental data used to fit the model. Should significant curvature exist, then the toxic load model is not appropriate, but it is still superior to Haber's Law in modeling the data in any event.

In the present protocol, 3-separate exposure-durations were used: 10, 60 and 180 min. Only 5 to 7 minipigs of each gender were tested for each exposure-duration:

Total No. of Minipigs Tested: 37
Total No. of Males Tested: 19
Total No. of Females Tested: 18

Statistical analysis routines contained within Minitab® version 13 (Minitab, Inc., State College PA), as well as an in-house developed spreadsheet program, were used for data analysis.

## 3. RESULTS

### 3.1 Minipigs Tested.

A total of 39 pigs were exposed to concentrations of GB to assess for miosis. One animal was disqualified from the studies due to a problem in the left eye. The results of another pig were discounted due to a problem with the generation of GB during the exposure. Therefore, 37 minipigs (19 males and 18 females) were used in the calculations of $EC_{50}$s over the 3 different exposure-durations. An additional female pig was used as an air control. At the time of the surgeries, the 19 males weighed an average of 10.92 kg ± 0.38 (SEM) kg and the 19 females, weighed an average of 11.17 ± 0.33 (SEM) kg each.

### 3.2 Median Effective Dosages and Time to Effect for Miosis.

Pupil areas were calculated from images (Figure 2) as described in section 2.9. Time-to-miosis (TM) was determined by plotting the pupil area versus time of GB exposure (Figures 5, 6, and 7). A minimum of 5-pre-exposure images were captured and the average of the pupil areas of these images were used as the baseline pupil area. Miosis is defined as pupil area 50% or less of the baseline pupil area. Table 2 gives exposure-durations and concentrations of GB exposures for male and female minipigs, the minimum post-exposure pupil area as a percent of the baseline pupil area, and whether or not the pigs developed miosis. Table 3 gives times to miosis for male and female minipigs that developed miosis. The $ECT_{50}$ values for miosis were estimated by maximum likelihood for male and female pigs at exposure-durations of 10, 60, and 180 min, and the results (with asymptotic 95% confidence intervals) are found in Table 4, as well as shown in Figures 8-12.

The MLE analysis used an assumed probit slope of 10 to estimate the $ECT_{50}$ separately for each gender and exposure-duration group. To determine the relationship between

pupil constriction based on area measurements and pupil constriction based on diameter measurements for a minipig's eye structure, a statistical model was constructed (see Appendix B). The $ECT_{50}$ values were also estimated based on pupil diameter, which means that miosis is defined as pupil diameter 50% or less of the baseline pupil diameter (Table 4). In general, the $ECT_{50}$'s on a diameter basis were greater than those on an area basis, but there are a few instances where they are equal in value (due to the way the quantal data came out) for a gender and exposure-duration group.

The TM data for the 17 minipigs (9 male and 8 female) exhibiting miosis on an area basis (see Table 3) were analyzed via linear regression, with the logarithm of TM (in minutes) being regressed against the logarithm of exposure concentration (EC) in $mg/m^3$. The TM value was found to equal $(8.52)(EC)^{-0.74}$ with an R-squared of 85.0%. The standard error of the exponent –0.74 was 0.08.

3.3 Statistical Models for the Probability of Miosis.

Several different models were used to fit the quantal data shown in Table 3 in order to model the probability of miosis as a function of EC, exposure-duration, and gender. The number of individuals used per gender-exposure-duration group is not enough to estimate the response distribution per each group. Instead, the response distribution was estimated using eqs 1 or 2 with all of the data grouped together into one large dataset of 37 minipigs (see Section 2.11).

Ordinary probit analysis on the total dataset did not produce stable or precise solutions. The standard errors of the fitted coefficients were too large (see Appendix C). Instead, ordinal regression was used to fit various response models (eq 1 and 2) to the data. To obtain ordinal data, the pupil area quantifications were classified into 4 categories with boundaries of 16%, 50%, and 84% of baseline pupil area. For ordinal regression on a diameter basis, Table B-2 (Appendix B) was used to establish the boundaries for 16%, 50% and 84% of baseline pupil diameter on an area basis (6.31, 32.0, and 74.3% of baseline area, respectively). The exact ordinal scores used are listed in Appendix C, along with printouts of the MINITAB® results.

A total of 6 different combinations of model terms (the same for both area and diameter basis) were used, as listed in Table 5. Exposure-duration is treated as a factor (*Time*) in Models A1, A2, A3, D1, D2 and D3, and as a continuous covariate ($\log T$) in the others (A4, A5, A6, D4, D5 and D6). The fits for Models A2, A3, A5 and A6 are compared to the MLE estimates for median effective dosages for miosis (area basis) in males and females in Figures 9-12.

Possible gender effects were tested for in Models A1, A2, A4, A5, D1, D2, D4 and D5, and for the most part, the effects were of borderline statistical significance at best. For individual gender-duration groups, the statistical significance between males and females at the exposure-duration of 180 min had a p-value of 0.031 (Model A1). For all the duration groups considered as a whole (Model A2), a p-value of 0.063 was obtained for the gender term, which is probably driven by the largest of the differences in individual gender-duration groups (180 min).

In practical terms, there is only about a 10% difference in the model fits for the miosis $ECT_{50}$s between the genders (when gender is in the model) with the females being more sensitive.

The time dependence of miosis (Haber's Law, TL model or other) was tested for in Models A4, A5, A6, D4, D5 and D6. The value of the TL exponent ($n = k_C / k_T$) was essentially independent of the model used: $1.32 \pm (2)(0.09)$ or a 95% confidence interval of 1.14 to 1.50. Since this interval does not overlap one, Haber's Law is not an appropriate time dependence model for this dataset. Potential curvature in the data was evaluated by inserting a $(\log T)^2$ term into Model A6 (see Appendix C), and this term was found to be statistically significant (p-value of 0.001). Thus, the TL model is not fully applicable either (though it is still better than Haber's Law in modeling the time dependence in this instance).

The gender term was not statistically significant for any of the models where exposure-duration was treated as a covariate. Thus, the best TL model fits for predictions of 50% reduction (of pupil area and diameter) are Models A6 and D6 (with no terms or interactions containing gender):

$$Y_N = (0.4286) + (6.402)(\log_{10} C) + (4.803)(\log_{10} T) \text{ (Area Basis—A6)} \quad (4)$$

$$Y_N = (1.0067) + (8.154)(\log_{10} C) + (6.184)(\log_{10} T) \text{ (Diameter Basis—D6)} \quad (5)$$

The steepness of the dose/response curve (as represented by the probit slope (concentration), $k_C$) was initially assumed to equal 10 for the purposes of executing the up-down experimental design used in this study (Section 2.11). The steepest probit slopes (concentration) were found when exposure-duration was treated as a factor. The probit slope values (with duration as a covariate—see eqs 3 and 4) are depressed in comparison to the "true" value due to the significant curvature found in the data (which is not properly accounted for in any of the duration (covariate) models (A4, A5, A6, D4, D5 and D6)). Instead, the presence of curvature is implicitly accounted for when duration is treated as a three-level factor (Models A1, A2, A3, D1, D2 and D3). From these models, the probit slope (concentration) values range from 14.5 to 24.6 (area basis) and 15.8 to 21.7 (diameter basis). When the standard errors for the probit slope values are considered, there is no statistically significant evidence from 4 of the 6 models (A2, A3, D2 and D3) to reject the original assumption that the probit slope equals 10. For the other 2 models (A1 and D1), the model fits suggest that the slope is actually steeper than 10. However, with the paucity of quantal data, the fits from Models A1 and D1 could be considered suspect due to possible overfitting of the data. Thus, it is best to state that the ordinal logistic regression results do not disprove the original assumption that the probit slope equals 10.

### 3.4 GB Regeneration and Cholinesterase Inhibition.

Results of changes in ChE activity and regenerated GB in RBC and plasma fractions of the blood samples extracted throughout the nerve agent exposures will be included in a separate report.

# 4. DISCUSSION

## 4.1 Infrared Pupillometry.

The improved contrast of a bright pupil image against a dark background (iris) is created using a beam of IR light reflected from off the retina. Dynamic pupillometers have been previously used to calculate real-time pupil area changes. While the eye of a pig has many anatomical and physiological similarities to the human eye one major difference exists.[20] The pig's pupil constricts into a rough ellipse rather than a circle making a simple measurement of pupil diameter an inaccurate method of assessing pupil constriction. Additionally, the measurement of pupil area is preferred over diameter since area is directly proportional to the quantity of light entering the eye, regardless of pupil shape.[21] However, in order to make limited comparisons between this study and those that have previously calculated $ECT_{50}$ values based on diameter measurements, a mathematical model was constructed. This model assesses the relative change between the horizontal and vertical radii in a constricting pig pupil and creates a mathematical formula for conversion of area measurements to diameter measurements.

## 4.2 Median Effective Dosages.

Mioduszewski and colleagues investigated how $ECT_{50}$ values changed with the duration of exposure.[7] Reports by Mioduszewski et al. and others are consistent with the present study in which CT (miosis) is not constant over time when GB is the agent.[7,22] Evidence that this is also the case when GF is the nerve agent has recently been shown.[19] Both studies conducted the same 10- and 60-min nerve agent exposures performed in this study. The rats used in the GB study by Mioduszewski et al were as much as 3 times more sensitive to the nerve agent than the minipigs used in this study.[7] However, limited conclusions can be drawn between the studies because two different techniques were used for quantifying pupil constriction.

In his study, Johns estimated that a CT of approximately 4 $mg.min/m^3$ would result in 50% decrease in pupil diameter in humans based on exposures ranging from 2-20 min.[23] McKee and Woolcott reported the threshold for development of GB vapor induced miosis was 3.3 $mg.min/m^3$.[22] These estimates are not far from the results of the present study in which the $ECT_{50}$s for 10-min exposures in males and female pigs were 2.60 and 2.20 $mg.min/m^3$, respectively.

## 4.3 Extraneous Variables.

One important consideration when calculating the incidence of miosis is the actual measurement of miosis. In this study, the minipigs were classified as being positive for miosis if the pupil area decreased to 50% or less of the baseline measurements at any time during the exposure or during an extended monitoring period after the exposure had been concluded. The majority of studies investigating miosis estimate the extent of pupil constriction at some time after exposure has been terminated and the animal removed from the inhalation chamber. Had the methods for this study been altered to monitor and quantify the degree of pupil constriction only at a set time (30-40 min) after the conclusion of the exposure, the results would be somewhat different. All the animals receiving a 10-min GB exposure that were classified as

having 50% miosis with the method described in this study would still be positive for miosis under the alternate criteria. Indeed the majority of miosis in the 10-min exposures was not evident until some time after GB generation had terminated. Two minipigs (1 male, 1 female) receiving a 60-min GB exposure that were classified as positive for miosis in this study would not have been considered positive for miosis using the alternate criteria, which would have resulted in minor changes to the calculated $ECT_{50}$s for the 60-min exposures.

In reassessing the data from the 180-min exposures, it was evident that the characterization of whether or not an animal has miosis is especially contingent on the length of time it takes from the conclusion of the nerve agent exposure to when the pupil area is quantified. Pupil constriction, elicited by the low concentrations used during the 180-min GB exposures, was rapidly reversible. Indeed, the pigs recovered so rapidly that only one pig that was positive for miosis in the 180-min exposures would have been positive for miosis using the alternate criteria. With these results, calculating an $EC_{50}$ for miosis in either males or females would not have been possible because only one pig would have been classified as being positive for miosis. Had the alternate criteria been used for measuring the pupil areas 30 to 40 min after the exposure conclusion, significantly higher concentrations of GB would have had to be used for the 180-min GB exposures for more minipigs to be considered positive for miosis and $EC_{50}$ calculation. Thus, the possibility exists that pupil constriction estimation at a set time after the conclusion of the experiment, especially for low concentrations over an extended exposure-duration, may result in overestimation of $EC_{50}$ values.

The human pupil is never known to be truly at rest. Whether the pupil is undergoing small, rapid changes in size (pupillary unrest) or large rhythmic changes in size (hippus), it is certain that the pupil is in a dynamic state. Pupillary unrest occurs at all levels of illumination leading to changes in pupil diameter up to 1/3 of a millimeter.[24]

The frequency of hippus in humans has been reported to be on the order of 8-14 per min with the pupil diameter changing as much as 1 mm.[25] Hippus is believed to be the result of poor balance between sympathetic and parasympathetic control of the eye.[26] Indeed, it is known that the muscles of the eye are under both sympathetic and parasympathetic control.[27,28] Stimulation of the parasympathetic control results in muscle contraction and, thus, constriction of the pupil. Such is the case when the eye is subjected to nerve agent exposure and there is local inhibition of acetylcholinesterase. Alternately, stimulation of the sympathetic system results in pupil dilation.

This study has evidenced that the sympathetic system can transiently override the parasympathetically induced pupil constriction at the level of nerve agent vapor used. The stress caused by removing the minipig from the chamber almost always resulted in a transient increase in pupil area even after the pupil had decreased to pinpoint size (see Figures 5, 6 and 7 for examples). This observation is important when determining which method is used for measuring pupil constriction (the amount of stress on the animal inherent in the measurement technique). Obviously, any manipulation of the animal that results in stress may induce a corresponding sympathetic response, briefly confounding pupil measurement. Therefore, one of the major benefits of capturing real-time IR images is that data can be collected while a minipig is in a relaxed state. Indeed, pigs can become so relaxed that they have to be aroused during the longer

exposures with auditory stimulation in order to keep them from closing their eyes and falling asleep. Miosis elicited as the first noticeable effect of an inhalation exposure is conventionally believed to be caused by a direct effect on the eye rather than via systemic circulation. In fact, the simple act of using a bandage to cover the eyes of individuals being exposed to vapor GB has been shown to offer significant protection from GB induced pupil constriction.[29]
If some experimental animals were to close their eyes in order to sleep during exposure, the eye would have less direct exposure to the GB vapor. This precept may provide some insight into the differences in $ECT_{50}$ calculations observed for the longer exposure-durations in this and other studies.

## 4.4 Gender Differences.

In the studies by Mioduszewski and colleagues, statistically significant differences existed between female and male rats at the shortest (10-min) and longest (240-min) exposure-durations while the 60-min results were just below statistical significance ($p=0.023$). The same statistically significant differences in miosis $EC_{50}$s were evident for GF exposures over the same exposure-durations.[19] For both the GB and GF studies the females were more sensitive. A borderline statistically significant gender difference was found in this study for the 180-min exposures, again with the females being more sensitive. However, because both male and female pigs could not be simultaneously housed together, there was no true randomizing of the sexes during the testing. This may account for the statistical difference between the sexes at the longer exposure-duration. Despite this potential shortcoming, the results from this study support previous studies in which the difference between the male and female $EC_{50}$ values was greatest for the longest exposure-duration.[7]

# 5. CONCLUSIONS

The present study utilized infrared pupillometry to digitally capture images of pupils in real-time during the whole-body exposures of Gottingen minipigs to GB vapor. Ordinary probit analysis on the total dataset did not produce stable or precise solutions. Instead, ordinal regression was used to fit various response models to the data. Values for the concentrations resulting in miosis in 50% ($EC_{50}$) and the median effective dose, $ECT_{50}$ (the dosage at which 50% of the exposed individuals exhibit a specified biological response) were calculated for miosis in male and female minipigs exposed to GB vapor for 10-, 60- and 180-min. The $ECT_{50}$ associated with miosis was not constant over time as predicted by Haber's law. The value of the toxic load (TL) exponent was essentially independent of the model used: $1.32\pm$ or a 95% confidence interval of 1.14 to 1.50. Since this interval does not overlap one, Haber's Law is not an appropriate time dependence model for this dataset. The best TL model fits are Models A6 and D6. Potential curvature in the data was evaluated by inserting a $(\log T)^2$ term into Model A6 (see Appendix C), and this term was found to be statistically significant (p-value of 0.001). Thus, the TL model is not fully applicable either (though it is still better than Haber's Law in illustrating the time dependence in this instance).

It was found that the time-to-miosis was equal to (5.42) (Exposure-duration (min))$^{0.62}$ with a fit R-square of 92.7%. In other words, when pupil constriction does occur, it

does not appear (on average) until the post-exposure period for short exposure-durations (less than 85 min). For exposures over 85 min, the mean time to miosis will be less than the length of the exposure-duration. In one model (Model A1) there was a borderline statistical significance ($p=0.031$) between males and females at the exposure-duration of 180 min. In practical terms, there was only about a 10% difference in the model fits for the miosis $ECT_{50}$s between the genders (when gender is in the model) with the females being more sensitive.

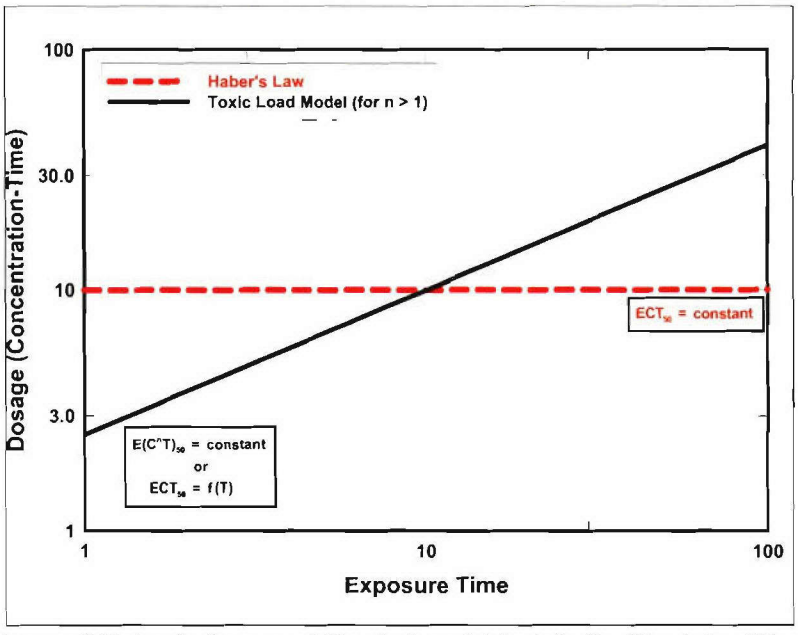


Figure 1. Comparison of Haber's Law and Toxic Load Models for Toxicity Time Dependence

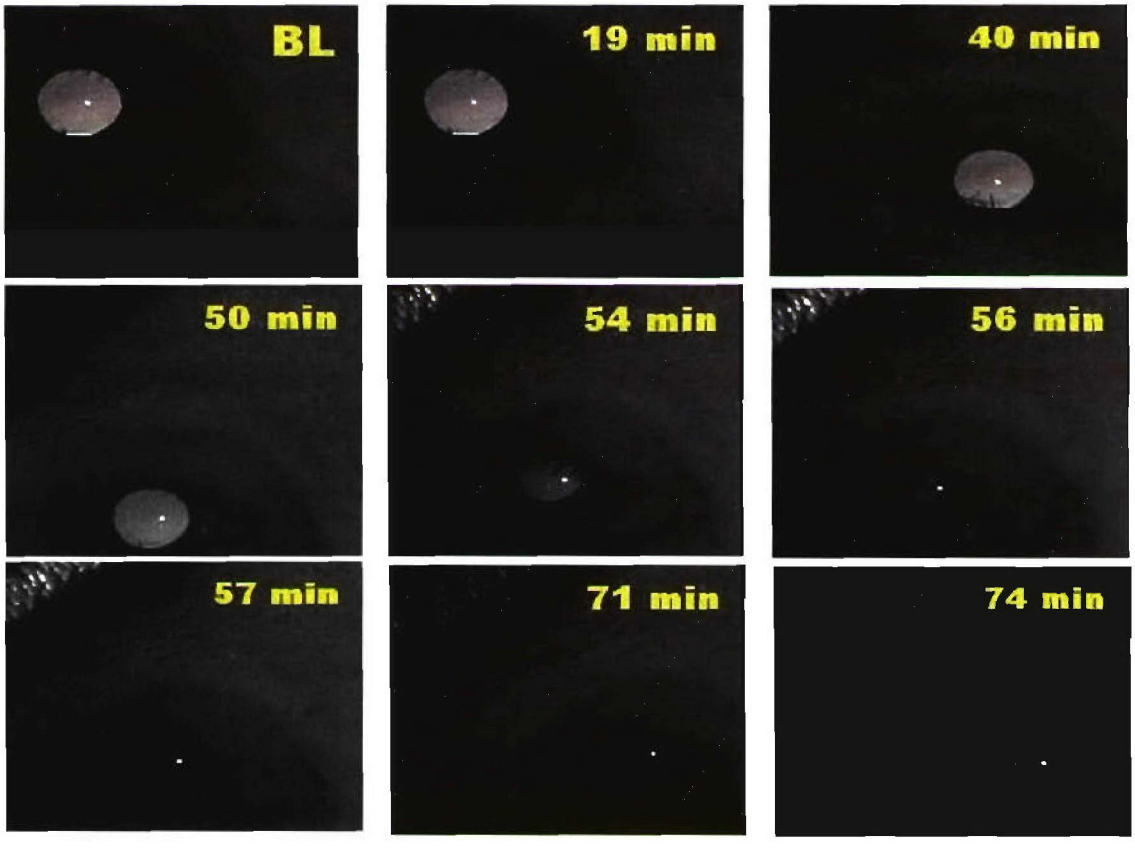


Figure 2. Infrared Images Showing the Progression of Pupil Constriction from Baseline to Complete Miosis during a 60-min Whole-Body 0.47-mg/m$^3$ GB Vapor Exposure in a Male Minipig

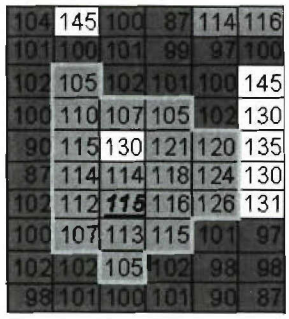


Figure 3. Border Location Determination around the Pupil

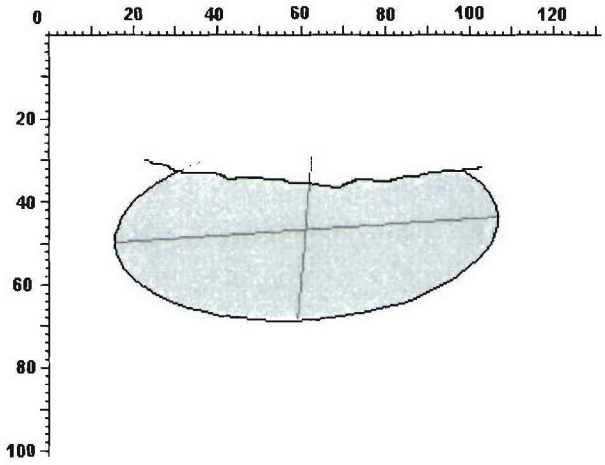


Figure 4. Graphic Representation of a Partially Obstructed Pupil

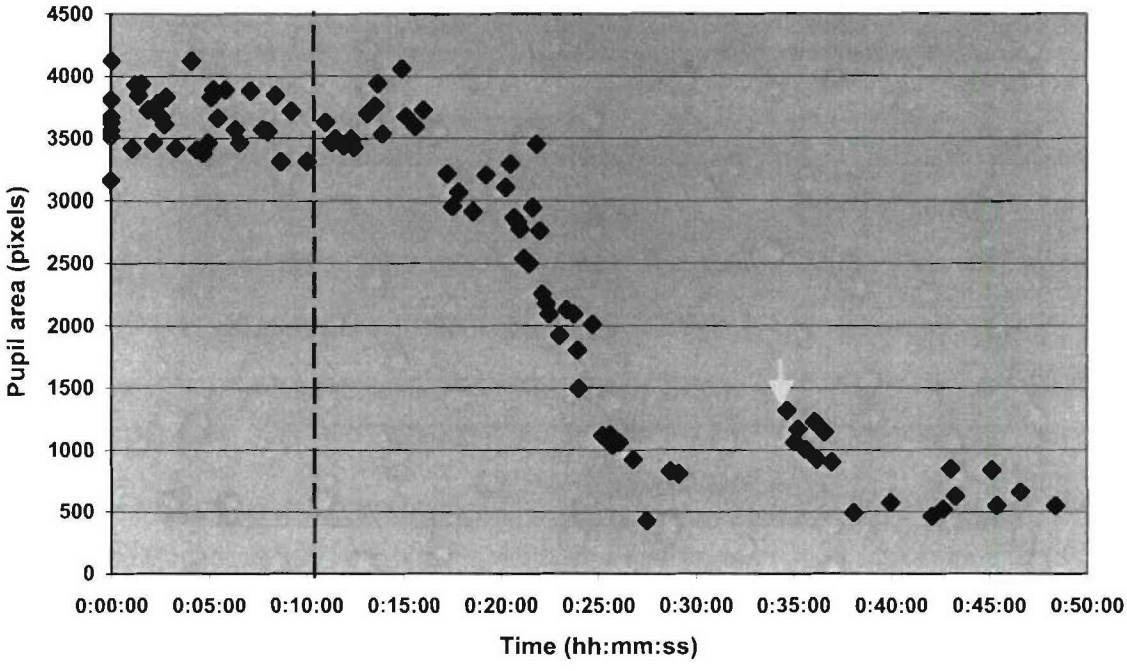


Figure 5. Pupil Area Versus Time of 10-Min GB Exposure

Each point represents the pupil area calculated from an image captured during a 10-min exposure of minipigs to 0.30-mg/m$^3$ GB. Points along the Y-axis are baseline measurements taken before GB vapor generation. The dotted line indicates the conclusion of GB generation. The arrow indicates pupil areas calculated from the first images taken after a pig was removed from the inhalation chamber.

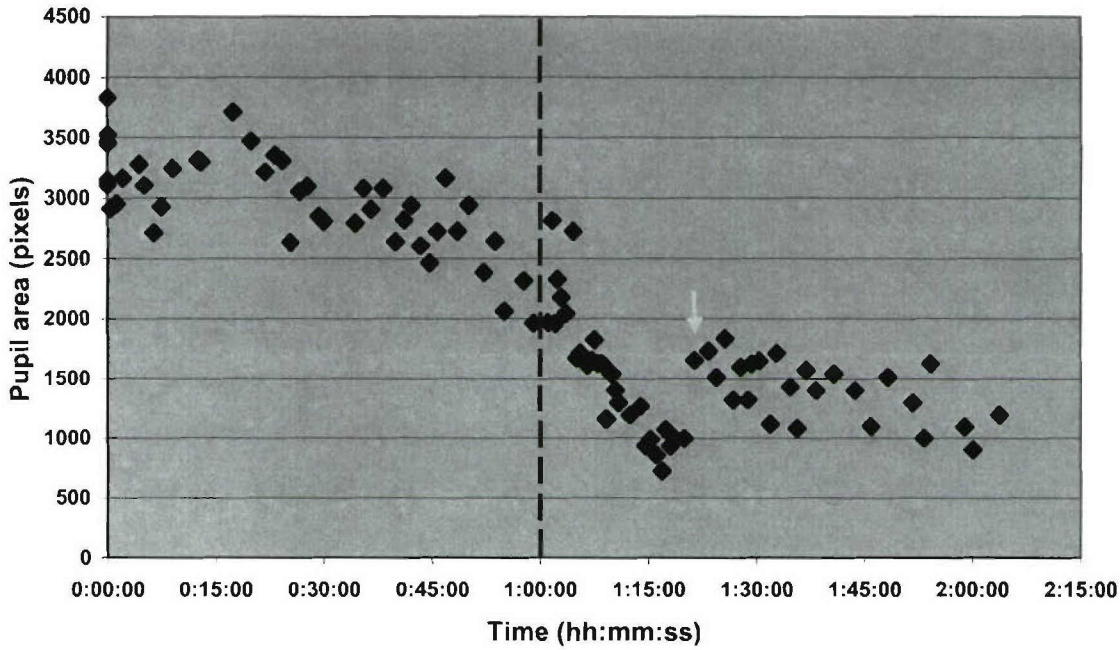


Figure 6. Graph of Pupil Area Versus Time of Exposure in a Minipig Exposed to GB Vapor for 60 Min

Each point represents the pupil area calculated from an image captured during a 60-min whole-body minipig exposure to 0.044-mg/m$^3$ GB. Points along the Y-axis are baseline measurements taken before GB vapor was generated. The dotted line indicates the conclusion of GB generation. The arrow indicates pupil areas calculated from the first images taken after a minipig was removed from the inhalation chamber.

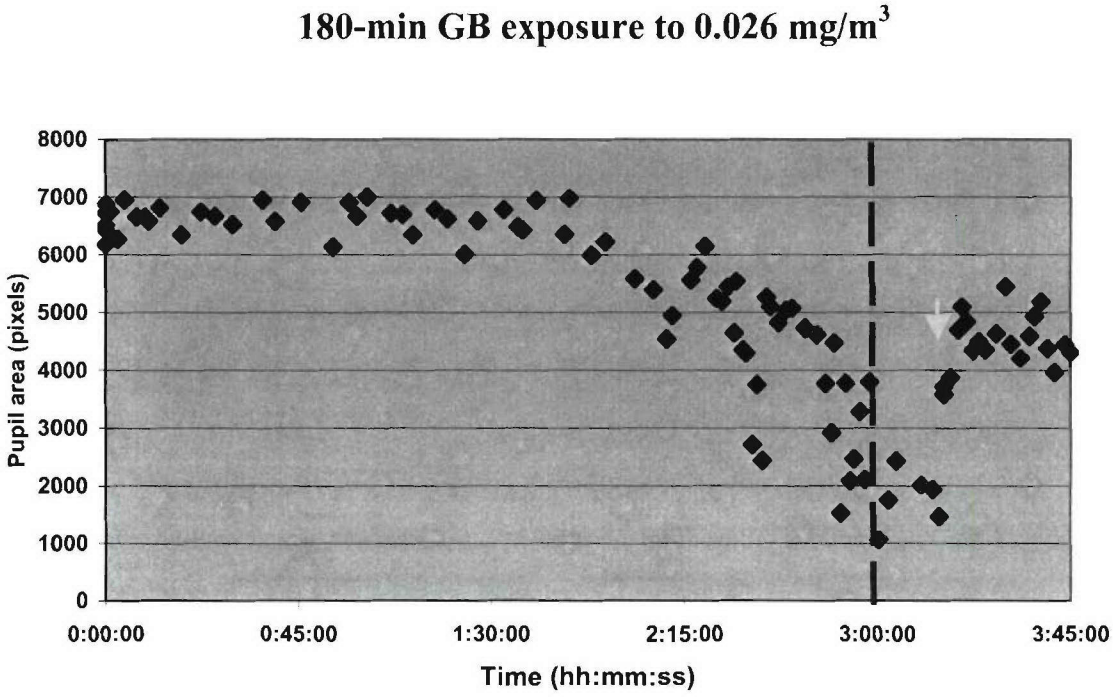


Figure 7. Graph of Pupil Area Versus Time of Exposure in a Minipig Exposed to GB Vapor for 180 Min

Each point represents the pupil area calculated from an image captured during a 180-min whole-body minipig exposure to 0.026-mg/m$^3$ GB. Points along the Y-axis are baseline measurements taken before GB vapor was generated. The dotted line indicates the conclusion of GB generation. The arrow indicates pupil areas calculated from the first images taken after a pig was removed from the inhalation chamber.

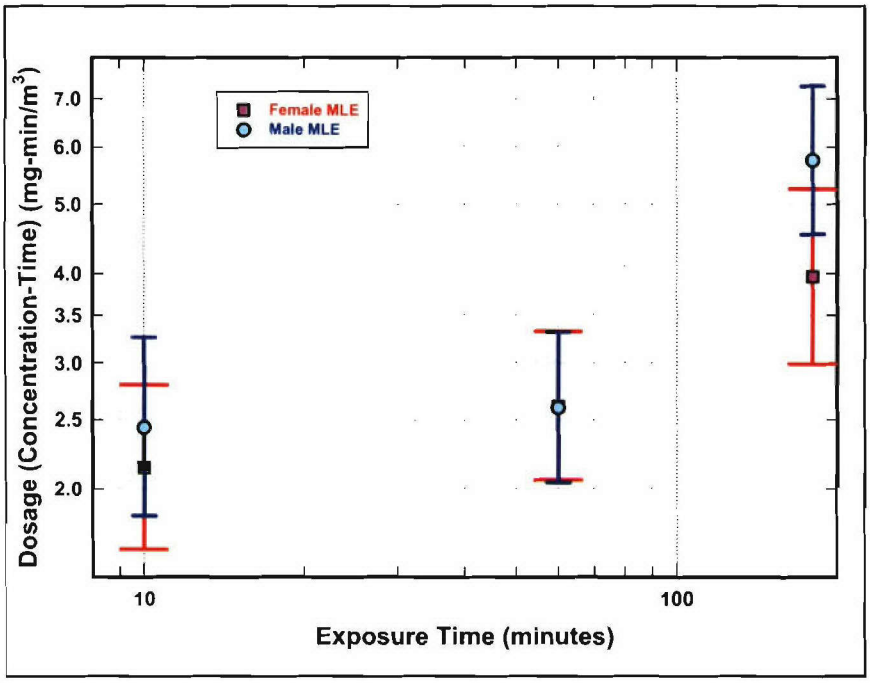


Figure 8. Miosis $ECT_{50}$ MLE Estimates for Male and Female Minipigs as a Function of Exposure-Duration

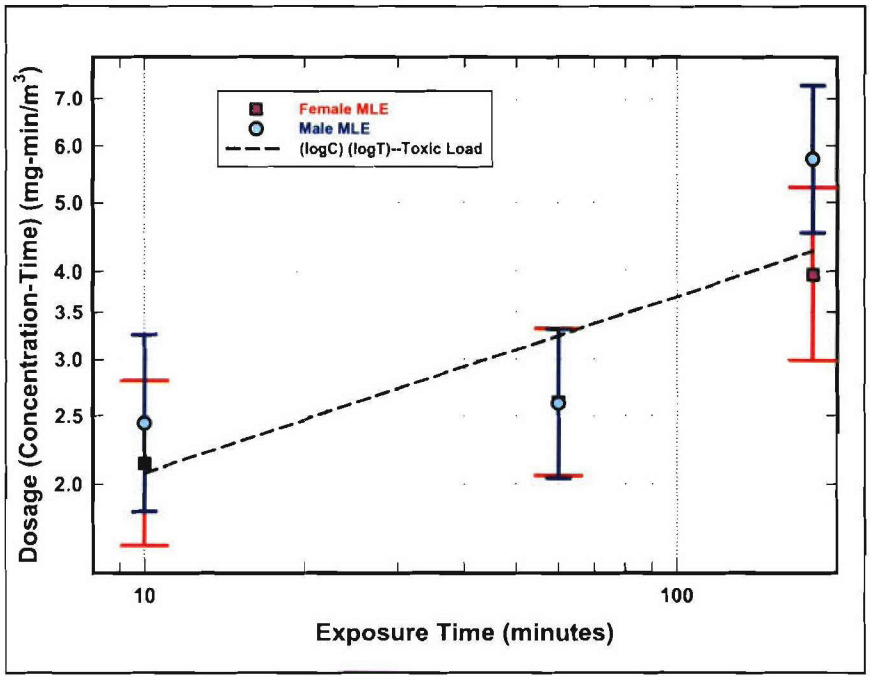


Figure 9. Toxic Load Fit (Model A6) of MLE $ECT_{50}$ Estimates for Minipigs without Gender as a Term

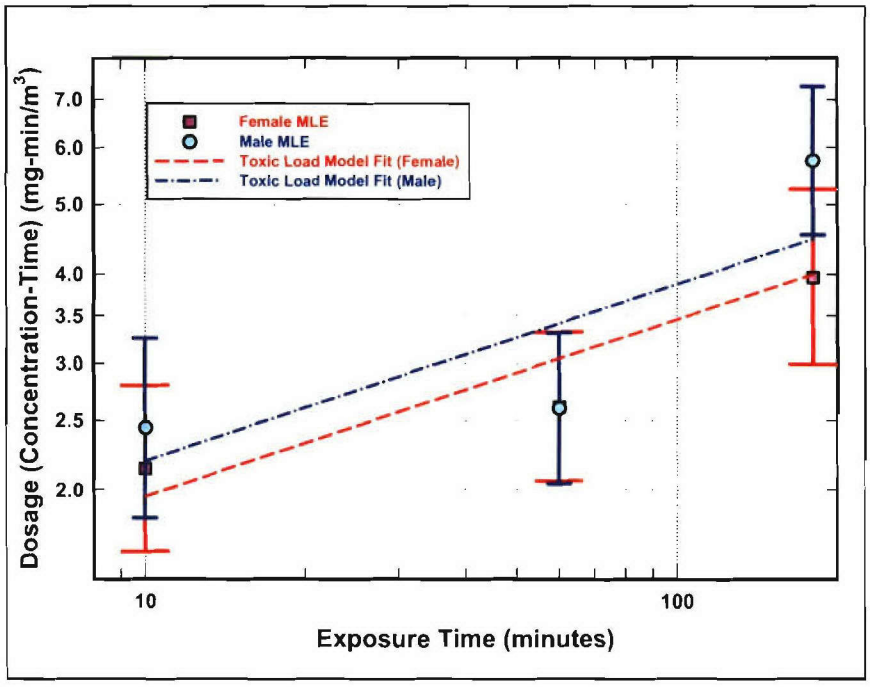


Figure 10. Toxic Load Fits (Model A5) of MLE $ECT_{50}$ Estimates for Male and Female Minipigs with Gender Included as a Term

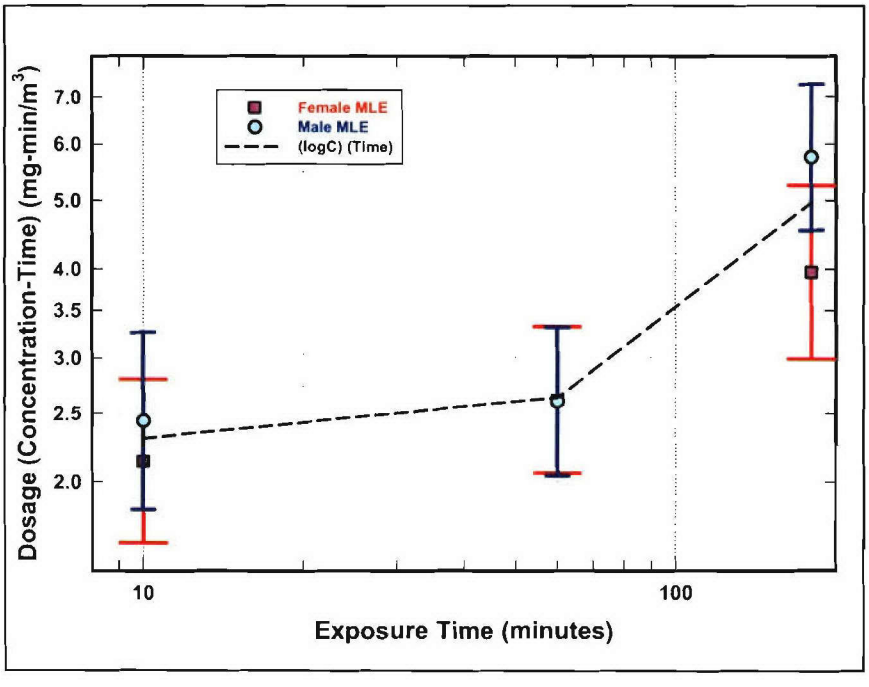


Figure 11. Toxic Load Fit (Model A3) of MLE $ECT_{50}$ Estimates for Minipigs without Gender as a Term

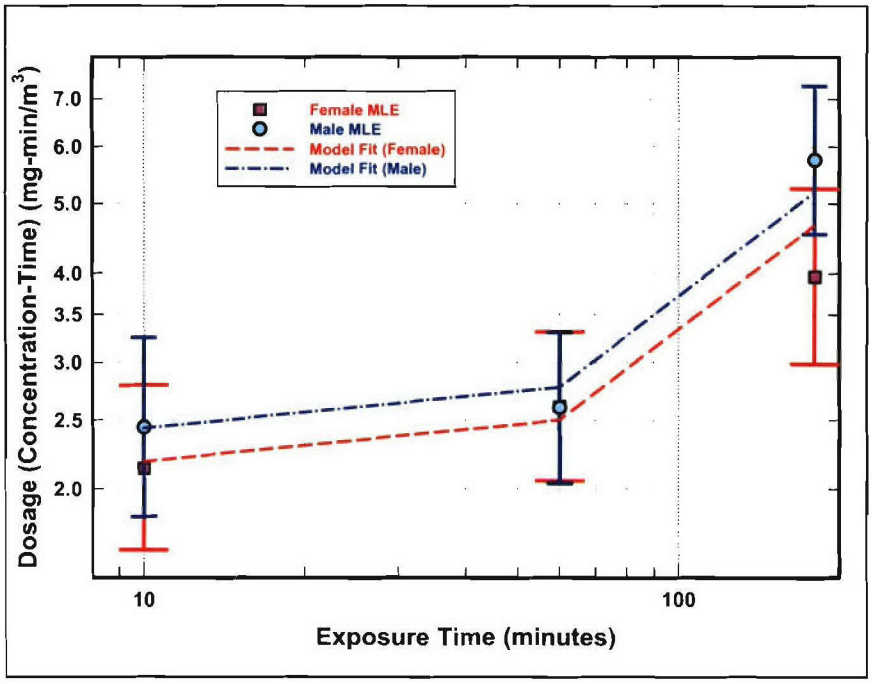


Figure 12. Toxic Load Fits (Model A2) of MLE $ECT_{50}$ Estimates for Male and Female Minipigs with Gender Included as a Term

Table 1. GB Analysis

| Compound | Mole % | Calculated Wt % |
|---|---|---|
| Methylphosphonofluoridic acid (Fluor Acid) | 0.3 | 0.2 |
| Diisopropyl methylphosphonate (DIMP) | 0.2 | 0.3 |
| Methylphosphonic difluoride (DF) | 0.2 | 0.2 |

Table 2. Durations and Concentrations of GB Exposures for Male and Female Minipigs, the Minimum Post-Exposure Pupil Area as a Percent of the Baseline Pupil Area, and Whether or Not the Pigs Developed Miosis (O = no miosis, X = miosis)

| Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Time (min.) | Conc. ($mg/m^3$) | CT | Area % | Miosis (Area) | Time (min.) | Conc. ($mg/m^3$) | CT | Area % | Miosis (Area) |
| 10 | 0.170 | 1.70 | 82 | O | 10 | 0.160 | 1.60 | 90 | O |
| 10 | 0.170 | 1.70 | 90 | O | 10 | 0.180 | 1.80 | 94 | O |
| 10 | 0.260 | 2.60 | 63 | O | 10 | 0.220 | 2.20 | 55 | O |
| 10 | 0.260 | 2.60 | 22 | X | 10 | 0.220 | 2.20 | 12 | X |
| 10 | 0.300 | 3.00 | 12 | X | 10 | 0.240 | 2.40 | 31 | X |
| 10 | 0.310 | 3.10 | 14 | X | 10 | 0.280 | 2.80 | 15 | X |
| 60 | 0.037 | 2.22 | 81 | O | 60 | 0.030 | 1.80 | 86 | O |
| 60 | 0.042 | 2.52 | 69 | O | 60 | 0.037 | 2.22 | 62 | O |
| 60 | 0.044 | 2.64 | 74 | O | 60 | 0.040 | 2.40 | 65 | O |
| 60 | 0.044 | 2.64 | 21 | X | 60 | 0.043 | 2.58 | 44 | X |
| 60 | 0.046 | 2.76 | 41 | X | 60 | 0.044 | 2.64 | 83 | O |
| 60 | 0.047 | 2.82 | 10 | X | 60 | 0.048 | 2.88 | 45 | X |
| 180 | 0.021 | 3.78 | 55 | O | 60 | 0.048 | 2.88 | 19 | X |
| 180 | 0.028 | 5.04 | 81 | O | 180 | 0.020 | 3.60 | 84 | O |
| 180 | 0.031 | 5.58 | 66 | O | 180 | 0.022 | 3.96 | 73 | O |
| 180 | 0.031 | 5.58 | 69 | O | 180 | 0.023 | 4.14 | 36 | X |
| 180 | 0.033 | 5.94 | 30 | X | 180 | 0.026 | 4.68 | 16 | X |
| 180 | 0.033 | 5.94 | 30 | X | 180 | 0.028 | 5.04 | 12 | X |
| 180 | 0.035 | 6.30 | 17 | X | | | | | |

Table 3. Durations of GB Exposure, Concentrations of GB Exposure and Times to 50% Miosis for Male and Female Minipigs that Developed Miosis

| Sex | Pig # | Exposure Duration (min.) | Conc. ($mg/m^3$) | Time to 50% Area (h:mm:ss) |
|---|---|---|---|---|
| M | 3 | 10 | 0.300 | 0:23:58 |
| M | 16 | 10 | 0.260 | 0:21:54 |
| M | 19 | 10 | 0.310 | 0:16:23 |
| M | 4 | 60 | 0.047 | 0:52:50 |
| M | 12 | 60 | 0.044 | 1:05:03 |
| M | 20 | 60 | 0.046 | 1:15:42 |
| M | 1 | 180 | 0.035 | 2:13:29 |
| M | 14 | 180 | 0.033 | 2:54:57 |
| M | 18 | 180 | 0.033 | 2:45:38 |
| F | 24 | 10 | 0.220 | 0:19:03 |
| F | 29 | 10 | 0.240 | 0:34:38 |
| F | 35 | 10 | 0.280 | 0:29:57 |
| F | 26 | 60 | 0.048 | 1:01:00 |
| F | 34 | 60 | 0.043 | 1:04:08 |
| F | 36 | 60 | 0.048 | 1:02:39 |
| F | 27 | 180 | 0.028 | 1:49:47 |
| F | 31 | 180 | 0.026 | 2:30:50 |
| F | 40 | 180 | 0.023 | 3:13:11 |

Table 4. Median Effective Concentrations and Dosages (as Estimated by Maximum Likelihood) with 95% Confidence Intervals for GB Exposure-Durations of 10, 60, and 180 Min

Area Basis

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| **Exposure-Duration (minutes)** | **$EC_{50}$** | **$ECT_{50}$** | **95% Limits** | **$EC_{50}$** | **$ECT_{50}$** | **95% Limits** |
| **10** | **0.244** | **2.44** | **1.83-3.26** | **0.214** | **2.14** | **1.64-2.80** |
| **60** | **0.043** | **2.60** | **2.04-3.31** | **0.044** | **2.61** | **2.06-3.32** |
| **180** | **0.032** | **5.75** | **4.53-7.29** | **0.022** | **3.96** | **2.99-5.25** |

Diameter Basis

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| **Exposure-Duration (minutes)** | **$EC_{50}$** | **$ECT_{50}$** | **95% Limits** | **$EC_{50}$** | **$ECT_{50}$** | **95% Limits** |
| **10** | **0.244** | **2.44** | **1.83-3.26** | **0.214** | **2.14** | **1.64-2.80** |
| **60** | **0.048** | **2.89** | **2.26-3.70** | **0.056** | **3.33** | **2.48-4.46** |
| **180** | **0.032** | **5.75** | **4.53-7.29** | **0.025** | **4.56** | **3.44-6.07** |

Table 5. Probit Slopes and Toxic Load Exponents Obtained from Various Ordinal Logistic Regression Model Fits

| ID | Basis | Terms in Model | $k_C$ | SE(C) | $k_T$ | SE(T) | $n$ | SE(n) |
|---|---|---|---|---|---|---|---|---|
| A1 | Area | LogC Time Sex Time*Sex | 24.6 | 5.3 | --- | --- | --- | --- |
| A2 | Area | LogC Time Sex | 17.5 | 3.8 | --- | --- | --- | --- |
| A3 | Area | LogC Time | 14.5 | 3.3 | --- | --- | --- | --- |
| A4 | Area | LogC LogT Sex LogT*Sex | 7.4 | 2.0 | 5.6 | 1.6 | 1.32 | 0.08 |
| A5 | Area | LogC LogT Sex | 7.0 | 2.0 | 5.2 | 1.5 | 1.33 | 0.09 |
| A6 | Area | LogC LogT | 6.4 | 1.8 | 4.8 | 1.4 | 1.33 | 0.10 |
| D1 | Diameter | LogC Time Sex Time*Sex | 21.7 | 5.1 | --- | --- | --- | --- |
| D2 | Diameter | LogC Time Sex | 17.8 | 4.0 | --- | --- | --- | --- |
| D3 | Diameter | LogC Time | 15.8 | 3.6 | --- | --- | --- | --- |
| D4 | Diameter | LogC LogT Sex LogT*Sex | 8.7 | 2.2 | 6.6 | 1.7 | 1.32 | 0.08 |
| D5 | Diameter | LogC LogT Sex | 8.6 | 2.2 | 6.5 | 1.7 | 1.32 | 0.08 |
| D6 | Diameter | LogC LogT | 8.2 | 2.1 | 6.2 | 1.6 | 1.32 | 0.08 |

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# APPENDIX A:
# ONE PARAMETER PROBIT ANALYSIS AND THE METHOD OF MAXIMUM-LIKELIHOOD ESTIMATION

## A1.0 INTRODUCTION

Traditionally, median effective dosages are determined via the use of probit analysis.[1,2] In conventional probit analysis, two parameters (the median effective dosage and the probit slope) are estimated simultaneously from experimental quantal data using the method of maximum likelihood estimation (MLE).[3-5] However, if there is an insufficient amount of quantal data available, solution instability is likely to occur when trying to solve for the two parameters. In this case, one of the two parameters (usually the probit slope) must be fixed at a set value in order to obtain a stable solution. This is an underlying principle of the up-down method for estimating median effective stresses and dosages.[6]

The following is an example of an one-parameter probit analysis applied to the male minipig GB miosis data for 10-min exposures derived from the present study.

## A2.0 MLE ALGORITHM OF ONE-PARAMETER PROBIT ANALYSIS

For each trial condition, $i$, there is a likelihood, $L_I$, of the observed result occurring:

$$L_i = \pi_i^{x} (1 - \pi_i)^{n-x} \tag{A1a}$$

$$\log_e(L_i) = x[\log_e \pi_i] + (n-x)\,[\log_e (1-\pi_i)] \tag{A1b}$$

where $\pi_i$ is the event probability for test condition $i$, $n$ is the number of independent trials under the $i$-th condition, and $x$ is the number of successes in $n$ trials under the $i$-th condition. The likelihoods for all test conditions are then multiplied together to arrive at the likelihood, $L$, which is the largest $\pi_I$ value supported by the quantal data. This set of values is the MLE, denoted $\hat{\pi}_i$.[3] For ease of calculation, the natural logarithm of the likelihood is often used.

In toxicology, the values of the individual $\pi_i$s are a function of the applied dosages, $(CT)_i$, used in an experiment and their respective distances from the median effective dosage, $ECT_{50}$. This is reflected in the following definition of the standard normal random variable, $Z_i$, as applied towards the present problem:

$$Z_i = \frac{\{\log(CT)_i - \log(ECT_{50})\}}{\sigma} \tag{A2}$$

where $\sigma$ is the inverse of the probit slope($m$). Tables of cumulative probabilities (event probabilities $\pi_i$) and their corresponding Z values are found in standard statistical textbooks or

obtained using statistical software. The 50% response level (or $\pi = 0.5$) corresponds to a $Z$ value of zero. The MLE estimate for $\log(ECT_{50})$ is the value of $\pi$ that maximizes $L$.

Thus, the following algorithm is used to estimate the $ECT_{50}$:

(1) Set the probit slope equal to some fixed value for the duration of the algorithm.

(2) Make an initial guess for $ECT_{50}$.

(3) Using eq A2, calculate $Z_i$ and $\pi_i$ for each test condition i, exposed to $(CT)_i$.

(4) Using eq A1, calculate the individual likelihoods, $L_i$.

(5) Multiply the $L_i$ together to estimate the total likelihood, $L$.

(6) Check to verify whether the maximum value of $L$ has been obtained. If not, go back to Step (3) with a new guess for the $ECT_{50}$.

To reach convergence at the value of $ECT_{50}$ that maximizes $L$, a Newton-Raphson (or Newton's Method) algorithm (or similar procedure) can be used.[3,7] After the final $ECT_{50}$ estimate is obtained, there are three common and general methods for obtaining approximate confidence limits for the estimate:[3] Wald test, likelihood-ratio test, and the score (or Lagrange-multiplier) test. These approximations grow more accurate as the sample size gets larger.

In the present study, the Wald test was used to calculate confidence limits. These limits from the Wald test can be readily obtained from calculations performed as part of the Newton-Raphson algorithm used for finding the maximum value for $L$. However, the likelihood-ratio test required additional Newton-Raphson algorithm iterations.

In the present study, the following equation was used (based on the Wald test) to calculate the 95% asymptotic confidence interval for the final MLE estimate of the $ECT_{50}$:[3]

$$\hat{\alpha} - \frac{(1.96)}{\sqrt{\frac{-d^2 \log_e L(\hat{\alpha})}{d\alpha^2}}} \leq \hat{\alpha} \leq \hat{\alpha} + \frac{(1.96)}{\sqrt{\frac{-d^2 \log_e L(\hat{\alpha})}{d\alpha^2}}} \qquad \text{(A3)}$$

where $\alpha$ equals the parameter of interest (*i*.e. $ECT_{50}$) and $\hat{\alpha}$ is the MLE estimate for the parameter. The second derivatives in eq A3 are easily evaluated (through numerical methods) as part of the Newton-Raphson algorithm used for finding the maximum value for $L$ (by locating where the first derivative, $(d[\log_e(L)] / d[\alpha])$, equals zero).

## A3.0 EXAMPLE OF APPLICATION OF ONE-PARAMETER PROBIT ANALYSIS

The following are the quantal miosis data for the 10-min exposures of the male minipig to GB vapor (from Appendix C). An individual pig is considered to have miosis if greater than 50% pupil area reduction occurred anytime after the start of the exposure run. Dosage is in units of mg-min/m$^3$.

**Table A1: Male Minipig GB Miosis Quantal Data (Area Basis) (10 Minute Exposure-Duration)**

| Individual | Dosage (CT) | $\log_{10}$(CT) | Miosis |
|---|---|---|---|
| 1 | 1.70 | 0.23045 | No |
| 2 | 1.70 | 0.23045 | No |
| 3 | 2.60 | 0.41497 | No |
| 4 | 2.60 | 0.41497 | Yes |
| 5 | 3.00 | 0.47712 | Yes |
| 6 | 3.10 | 0.49136 | Yes |

For this example, test condition $i$ will only have one test subject (though for CTs of 1.7 and 2.6 there were two individuals each). So, for eqs A1a and A1b, $n$ will equal one for each test condition.

Steps (1) and (2): Probit Slope and Initial Guess for $\log_{10}(ECT_{50})$ for Iteration One

For Step (1) of the algorithm, the probit slope will be set equal to 10, which was used as the basis for the up and down method employed in the present study. For Step (2), the initial guess for the $\log_{10}(ECT_{50})$ is 0.41.

Step (3): Calculation of $Z_i$'s and $\pi_i$ for Iteration One Using Eq A2

$$Z_1 = [(0.23045) - (0.41)] / (1/10) = (-1.7955) \Longrightarrow \pi_1 = (0.03629)$$

$$Z_2 = [(0.23045) - (0.41)] / (1/10) = (-1.7955) \Longrightarrow \pi_2 = (0.03629)$$

$$Z_3 = [(0.41497) - (0.41)] / (1/10) = (0.04973) \Longrightarrow \pi_3 = (0.51983)$$

$$Z_4 = [(0.41497) - (0.41)] / (1/10) = (0.04973) \Longrightarrow \pi_4 = (0.51983)$$

$$Z_5 = [(0.47712) - (0.41)] / (1/10) = (0.67121) \Longrightarrow \pi_5 = (0.74896)$$

$$Z_6 = [(0.49136) - (0.41)] / (1/10) = (0.81362) \Longrightarrow \pi_6 = (0.79207)$$

Steps (4) and (5): Calculation of $L_i$'s and $L$ for Iteration One Using Eq A1

$$\log_e(L_1) = (0)(\log_e(0.03645)) + (1-0)(\log_e(1-0.03645)) = (-0.03696)$$

$$\log_e(L_2) = (0)(\log_e(0.03645)) + (1-0)(\log_e(1-0.03645)) = (-0.03696)$$

$$\log_e(L_3) = (0)(\log_e(0.51983)) + (1-0)(\log_e(1-0.51983)) = (-0.73362)$$

$$\log_e(L_4) = (1)(\log_e(0.51983)) + (1-1)(\log_e(1-0.51983)) = (-0.65425)$$

$$\log_e(L_5) = (1)(\log_e(0.74896)) + (1-1)(\log_e(1-0.74896)) = (-0.28907)$$

$$\log_e(L_6) = (1)(\log_e(0.79207)) + (1-1)(\log_e(1-0.79207)) = (-0.23311)$$

Sum of the above $\log_e(L_i)$'s (or $\log_e(L)$) equals (-1.98405)

Step (6): Check for Convergence on Maximum $L$ Value and New Guess for $\log(ECT_{50})$ for Iteration Two

After the first iteration, convergence was not achieved using a Newton-Raphson algorithm. The algorithm returns a value of (0.3875) for the next guess for $\log_{10}(ECT_{50})$. Convergence was reached at the fourth iteration. The final estimate for $\log_{10}(ECT_{50})$ is (0.3873) (or $(ECT_{50}) = 2.44$ mg-min/m$^3$), and the final $\log_e(L)$ value was (-1.92089). The denominators of eq A3 were found to equal the square root of 244.6. With this value for the denominators, the corresponding 95% asymptotic confidence interval for the MLE estimate of $\log_{10}(ECT_{50})$ equals (0.262 to 0.513), or for $ECT_{50}$, the interval is (1.83 to 3.26 mg-min/m$^3$).

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# APPENDIX B:
# CONVERSION OF MIOSIS MEASUREMENTS FROM AN AREA BASIS TO A DIAMETER BASIS

Past miosis studies concentrated on the measurement of pupil diameters, not pupil areas. In order to compare the results of the present study (performed on an area basis) with these past studies, the area measurements need to be properly converted. If swine eyes were circular, the conversion would be simple:

$$\left[\frac{d_{pre}}{d_{post}}\right] = \sqrt{\frac{A_{pre}}{A_{post}}} \qquad \text{(B1)}$$

where $d_{pre}$ and $d_{post}$ are the pre and post diameters of the pupil and $A_{pre}$ and $A_{post}$ are the pre and post areas of the pupil, respectively. However, the swine's pupil is elliptical in shape, with radii of *(a)* (major axis--horizontal axis for the swine) and *(b)* (minor axis--vertical axis for the swine). To convert from an area to linear basis, the relationship between $a$ and $b$ must be observed under varying degrees of miosis.

Pupil size data from six individual swine trials (four from lethality trials and two from miosis trials) were examined. Linear regression was used to estimate the *(a)* versus *(b)* relationship for each pig:

$$a = mb + k \qquad \text{(B2)}$$

with $a$ and $b$ being the radii, and $m$ and $k$, the fitted coefficients. The coefficients for the fitted lines (using eq B2 are listed in Table B-1 (along with the corresponding square correlation coefficient (R-sq) for the fit), with the units of the radii ($a$ and $b$) in pixels, as well as the pre-exposure axes and area values for the individual swine.

Table B-1: Linear Regression Results for Major ($a$) versus Minor ($b$) Axes Changes in the Swine as a Result of a Miotic Response

| Fitted Coefficients | | | | Pre-Exposure Values | | |
|---|---|---|---|---|---|---|
| **Pig ID** | ***k*** | ***m*** | **R-sq** | ***a*** **(pixels)** | ***b*** **(pixels)** | **Area (sq. pixels)** |
| **1** | **15.1080** | **0.7354** | **92.3** | **39.2** | **32.8** | **4042** |
| **19** | **11.6420** | **0.9420** | **92.3** | **47.4** | **38.0** | **5663** |
| **43** | **11.2990** | **1.0019** | **90.5** | **48.5** | **37.1** | **5649** |
| **44** | **10.1750** | **0.8956** | **95.9** | **42.5** | **36.1** | **4821** |
| **45** | **14.5890** | **0.8057** | **92.8** | **49.2** | **43.0** | **6651** |
| **50** | **11.0090** | **0.9123** | **96.4** | **39.9** | **31.7** | **3976** |

The major axis does not decrease past a certain point in a swine (as represented by the fitted coefficient $k$ in Table B-1). The minor axis decreases to zero at pin-point (or complete) miosis. The following procedure was used to convert from an area to a diameter basis.

(1) Multiply the pre-exposure value of *(b)* for each pig by 0.16, by 0.50, and by 0.84 to create a set of three new *(b)* values for each pig.

(2) Use eq B2 to calculate new *(a)* values from the new *(b)* values.

(3) Calculate areas from the new *(a)* and *(b)* values using the formula: Area = $\pi ab$.

(4) Calculate the individual swine pupil area ratios (post/pre) for each diameter ratio examined (0.16, 0.50 and 0.84). Take an average value of the area ratios calculated for a particular diameter ratio. The results are summarized in Table B-2.

Table B-2: Corresponding Diameter and Area Ratios in the Swine as a Result of a Miotic Response

| | Area Ratio | |
|---|---|---|
| **Diameter Ratio** | **Swine Eye** | **Circular Eye** |
| **0.16** | **0.0631** | **0.0256** |
| **0.50** | **0.320** | **0.250** |
| **0.84** | **0.743** | **0.706** |

With Table B-2, it is possible to compare the present study to previous miosis studies in which miosis was defined by a diameter ratio. If a diameter ratio (post/pre) of 0.5 had been used to define miosis, the equivalent area ratio in the present study would be 0.32.

# APPENDIX C:
# PROBIT ANALYSIS AND ORDINAL LOGISTIC REGRESSION PRINTOUTS FROM MINITAB®

## C1.0 INTRODUCTION

Two types of statistical analyzes were used in MINITAB® on the total dataset (both genders and all three exposure-durations): traditional probit analysis (Section C2.0) and ordinal logistic regression with a normit link function (Section C3.0). In the present study, both methods produced approximately the same estimates for median effective dosages for the six gender-time groups. However, ordinal logistic regression was able to estimate the steepness of the dose-response curve for percent of individuals showing miosis (as represented by the probit slope), whereas the probit analysis could not.

With traditional probit analysis, the use of only 37 subjects divided among six exposure groups did not provide sufficient information for estimating the probit slope (as demonstrated in the MINITAB® printouts in Section C2.0). Ordinal logistic regression can use more information from the same dataset than can probit analysis, since ordinal regression can use responses of three or more levels (instead of the binary response used by probit analysis). So, ordinal regression was used to generate the final model fits for the quantal data.

Comments by the analyst about the printouts are preceded by [DRS].

NOMENCLATURE

| | |
|---|---|
| ID | Identification number of test animal |
| Gender | Factor: 1 for Male and 0 for Female |
| Sex | Covariate: 1 for Male and -1 for Female |
| C | GB vapor concentration ($mg/m^3$) |
| T | Exposure-duration (minutes) |
| CT | Concentration-time ($mg\text{-}minute/m^3$) |
| logCT | Logarithm (Base 10) of CT |
| logC | Logarithm (Base 10) of C |
| logT | Logarithm (Base 10) of T |
| $ECT_{50}$ | Effective Concentration-time to cause effect in 50% of exposed individuals |
| PercentA | Percent of pre-exposure pupil area (minimum observed) |
| PercentD | Percent of pre-exposure pupil diameter (calculation explained in appendix B) |
| MiosisA | Miosis indicator for an exposed animal based upon pupil area constriction:<br>0 for pupil area constriction less than 50%<br>1 for pupil area constriction equal to or greater than 50% |
| MiosisD | Miosis indicator for an exposed animal based upon pupil equivalent diameter constriction:<br>0 for pupil equivalent diameter constriction less than 50%<br>1 for pupil equivalent diameter constriction equal to or greater than 50% |
| ScoreA | Response score of an exposed animal based upon pupil area constriction:<br>0 for range ($100 \geq PercentA \geq 84$) |

3 1 for range (84 > PercentA ≥ 50)
2 for range (50 > PercentA ≥ 16)
3 for range (16 > PercentA ≥ 0)

ScoreDResponse score based upon pupil equivalent diameter constriction:
0 for range (100 ≥ PercentA ≥ 74 or 100 ≥ PercentD ≥ 84)
1 for range ( 74 > PercentA ≥ 32 or 84 > PercentD ≥ 50)
2 for range ( 32 > PercentA ≥ 6 or 50 > PercentD ≥ 16)
3 for range ( 6 > PercentA ≥ 0 or 16 > PercentD ≥ 0)

Group: Gender--exposure-duration combinations:
F10: Female--10 min exposure-duration
M10: Male--10 min exposure-duration
F60: Female--60 min exposure-duration
M60: Male--60 min exposure-duration
F240: Female--240 min exposure-duration
M240: Male--240 min exposure-duration

Data Display

| Row | ID | Group | Conc | CT | PercentA | MiosisA | MiosisD | ScoreA | ScoreD |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 8 | M10 | 0.170 | 1.70 | 82 | 0 | 0 | 1 | 0 |
| 2 | 9 | M10 | 0.170 | 1.70 | 90 | 0 | 0 | 0 | 0 |
| 3 | 2 | M10 | 0.260 | 2.60 | 63 | 0 | 0 | 1 | 1 |
| 4 | 16 | M10 | 0.260 | 2.60 | 22 | 1 | 1 | 2 | 2 |
| 5 | 3 | M10 | 0.300 | 3.00 | 12 | 1 | 1 | 3 | 2 |
| 6 | 19 | M10 | 0.310 | 3.10 | 14 | 1 | 1 | 3 | 2 |
| 7 | 7 | M60 | 0.037 | 2.22 | 81 | 0 | 0 | 1 | 0 |
| 8 | 10 | M60 | 0.042 | 2.52 | 69 | 0 | 0 | 1 | 1 |
| 9 | 13 | M60 | 0.044 | 2.64 | 74 | 0 | 0 | 1 | 1 |
| 10 | 12 | M60 | 0.044 | 2.64 | 21 | 1 | 1 | 2 | 2 |
| 11 | 20 | M60 | 0.046 | 2.76 | 41 | 1 | 0 | 2 | 1 |
| 12 | 4 | M60 | 0.047 | 2.82 | 10 | 1 | 1 | 3 | 2 |
| 13 | 6 | M180 | 0.021 | 3.78 | 55 | 0 | 0 | 1 | 1 |
| 14 | 5 | M180 | 0.028 | 5.04 | 81 | 0 | 0 | 1 | 0 |
| 15 | 11 | M180 | 0.031 | 5.58 | 66 | 0 | 0 | 1 | 1 |
| 16 | 17 | M180 | 0.031 | 5.58 | 69 | 0 | 0 | 1 | 1 |
| 17 | 14 | M180 | 0.033 | 5.94 | 30 | 1 | 1 | 2 | 2 |
| 18 | 18 | M180 | 0.033 | 5.94 | 30 | 1 | 1 | 2 | 2 |
| 19 | 1 | M180 | 0.035 | 6.30 | 17 | 1 | 1 | 2 | 2 |
| 20 | 23 | F10 | 0.160 | 1.60 | 90 | 0 | 0 | 0 | 0 |
| 21 | 37 | F10 | 0.180 | 1.80 | 94 | 0 | 0 | 0 | 0 |
| 22 | 33 | F10 | 0.220 | 2.20 | 55 | 0 | 0 | 1 | 1 |
| 23 | 24 | F10 | 0.220 | 2.20 | 12 | 1 | 1 | 3 | 2 |
| 24 | 29 | F10 | 0.240 | 2.40 | 31 | 1 | 1 | 2 | 2 |
| 25 | 35 | F10 | 0.280 | 2.80 | 15 | 1 | 1 | 3 | 2 |
| 26 | 30 | F60 | 0.030 | 1.80 | 86 | 0 | 0 | 0 | 0 |
| 27 | 21 | F60 | 0.037 | 2.22 | 62 | 0 | 0 | 1 | 1 |
| 28 | 38 | F60 | 0.040 | 2.40 | 65 | 0 | 0 | 1 | 1 |
| 29 | 34 | F60 | 0.043 | 2.58 | 44 | 1 | 0 | 2 | 1 |
| 30 | 22 | F60 | 0.044 | 2.64 | 83 | 0 | 0 | 1 | 0 |
| 31 | 26 | F60 | 0.048 | 2.88 | 45 | 1 | 0 | 2 | 1 |
| 32 | 36 | F60 | 0.048 | 2.88 | 19 | 1 | 1 | 2 | 2 |
| 33 | 25 | F180 | 0.020 | 3.60 | 84 | 0 | 0 | 0 | 0 |
| 34 | 32 | F180 | 0.022 | 3.96 | 73 | 0 | 0 | 1 | 1 |
| 35 | 40 | F180 | 0.023 | 4.14 | 36 | 1 | 0 | 2 | 1 |
| 36 | 31 | F180 | 0.026 | 4.68 | 16 | 1 | 1 | 3 | 2 |
| 37 | 27 | F180 | 0.028 | 5.04 | 12 | 1 | 1 | 3 | 2 |

## C2.0 PROBIT ANALYSIS OF MIOSIS VERSUS DOSAGE AND GROUP

### C2.1 Miosis on Area Basis as a Function of CT and Group.

Probit Analysis: MiosisA versus CT, Group

```
Distribution:  Lognormal base 10

Response Information

Variable  Value       Count
MiosisA   1              18  (Event)
          0              19
          Total          37

Factor Information
Factor   Levels Values
Group         6 M10  M60  M180 F10  F60  F180

Estimation Method:  Maximum Likelihood

Regression Table
                      Standard
Variable        Coef      Error        Z      P
Constant      -48.98      23.99    -2.04 0.041
CT            118.03      57.78     2.04 0.041
Group
 M60          -0.777      1.286    -0.60 0.546
 M180         -40.75      20.00    -2.04 0.042
 F10           8.563      4.375     1.96 0.050
 F60          -0.193      1.210    -0.16 0.873
 F180         -22.71      11.22    -2.02 0.043
Natural
Response       0.000

Test for equal slopes:  Chi-Square = 4.5223,  DF = 5,  P-Value = 0.477
Log-Likelihood = -7.237

Multiple degree of freedom test

Term      Chi-Square    DF      P
Group          4.163     5  0.526

Goodness-of-Fit Tests

Method                Chi-Square    DF      P
Pearson                    5.741    23  1.000
Deviance                   6.157    23  1.000
```

[DRS] The steep probit slope (118) and its high standard error (57.8) indicate an unstable solution. The MINITAB routine had trouble fitting both slope and median dosage estimates. The estimated median effective dosages are probably reliable, but the slope estimate is not.

Listing of Median Effective Dosages

Male Swine: 0-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 2.6000 | 0.04495 | 2.3053 | 2.9324 |

Male Swine: 60-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 2.6397 | 0.04348 | 2.3370 | 2.9403 |

Male Swine: 180-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 5.7572 | 0.1253 | 4.9480 | 6.6979 |

Female Swine: 10-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 2.2000 | 0.03803 | 1.9505 | 2.4810 |

Female Swine: 60-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 2.6098 | 0.04169 | 2.3355 | 2.9170 |

Female Swine: 180-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 4.0490 | 0.09734 | 3.4253 | 4.7863 |

Potency Comparison between the Six Levels of Group

Note: If the 95% fiducial CI do not overlap the value of 1.0, then there is a statistically significant difference between the two group levels being compared.

Table of Relative Potency
Factor: Group

| Comparison | Relative Potency | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|
| M10 VS M60 | 1.0153 | 0.8538 | 1.1906 |
| M10 VS M180 | 2.2143 | 1.8249 | 2.6866 |
| M10 VS F10 | 0.8462 | 0.7137 | 1.0030 |
| M10 VS F60 | 1.0038 | 0.8522 | 1.1825 |
| M10 VS F180 | 1.5573 | 1.2673 | 1.9136 |
| M60 VS M180 | 2.1810 | 1.8161 | 2.6557 |
| M60 VS F10 | 0.8334 | 0.7106 | 0.9910 |
| M60 VS F60 | 0.9887 | 0.8486 | 1.1682 |
| M60 VS F180 | 1.5339 | 1.2610 | 1.8921 |
| M180 VS F10 | 0.3821 | 0.3149 | 0.4636 |
| M180 VS F60 | 0.4533 | 0.3758 | 0.5471 |

```
M180 VS F180      0.7033      0.5613      0.8813
F10  VS F60       1.1863      1.0073      1.3976
F10  VS F180      1.8405      1.4979      2.2617
F60  VS F180      1.5514      1.2690      1.8963
```

[DRS] The difference between the genders is statistically significant for the 180-min exposures.

C2.2 Miosis on Equivalent Diameter Basis as a Function of CT and Group.

Probit Analysis: MiosisD versus CT, Group

```
Distribution:  Lognormal base 10

Response Information

Variable  Value       Count
MiosisD   1              14  (Event)
          0              23
          Total          37

Factor Information

Factor   Levels Values
Group         6 M10  M60  M180 F10  F60  F180

Estimation Method:  Maximum Likelihood

Regression Table
                       Standard
Variable        Coef       Error        Z      P
Constant      -31.84       14.52    -2.19 0.028
CT             76.72       34.93     2.20 0.028
Group
 M60          -1.370       1.240    -1.10 0.269
 M180         -26.49       12.13    -2.18 0.029
 F10           5.571       2.810     1.98 0.047
 F60          -3.413       1.976    -1.73 0.084
 F180        -17.547       8.307    -2.11 0.035
Natural
Response       0.000

Test for equal slopes:  Chi-Square = 1.8495,  DF = 5,  P-Value = 0.870
Log-Likelihood = -8.125

Multiple degree of freedom test

Term      Chi-Square    DF      P
Group          4.822     5  0.438

Goodness-of-Fit Tests

Method          Chi-Square    DF      P
Pearson              4.782    23  1.000
Deviance             5.159    23  1.000
```

[DRS] The steep probit slope (76.7) and high standard error for the slope (34.9) indicate an unstable solution. The MINITAB routine is having trouble fitting both slope and median dosage estimates. The estimated median effective dosages are probably reliable, but the probit slope estimate is not.

Listing of Median Effective Dosages

Male Swine: 10-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 2.6000 | 0.06915 | 2.3164 | 2.9182 |

Male Swine: 60-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 2.7091 | 0.05421 | 2.5220 | 3.0104 |

Male Swine: 180-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 5.7567 | 0.1437 | 5.1485 | 6.3954 |

Female Swine: 10-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 2.1997 | 0.05813 | 1.9511 | 2.4549 |

Female Swine: 60-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 2.8804 | 0.07607 | 2.5826 | 3.2492 |

Female Swine: 180-min exposure-duration

| Percent | Percentile | Standard Error | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|---|
| 50 | 4.4022 | 0.2852 | 3.3367 | 5.8569 |

Potency Comparison between the Six Levels of Group

Table of Relative Potency

Factor: Group

| Comparison | Relative Potency | 95.0% Fiducial CI Lower | Upper |
|---|---|---|---|
| M10 VS M60 | 1.0420 | 0.9163 | 1.2258 |
| M10 VS M180 | 2.2141 | 1.8837 | 2.5858 |
| M10 VS F10 | 0.8460 | 0.7153 | 0.9907 |
| M10 VS F60 | 1.1079 | 0.9468 | 1.3112 |
| M10 VS F180 | 1.6932 | 1.2545 | 2.3046 |
| M60 VS M180 | 2.1249 | 1.8096 | 2.3966 |
| M60 VS F10 | 0.8120 | 0.6866 | 0.9189 |
| M60 VS F60 | 1.0632 | 0.9102 | 1.2143 |
| M60 VS F180 | 1.6250 | 1.1950 | 2.1540 |
| M180 VS F10 | 0.3821 | 0.3257 | 0.4466 |
| M180 VS F60 | 0.5004 | 0.4310 | 0.5913 |
| M180 VS F180 | 0.7647 | 0.5699 | 1.0416 |
| F10 VS F60 | 1.3095 | 1.1250 | 1.5573 |
| F10 VS F180 | 2.0013 | 1.4905 | 2.7373 |
| F60 VS F180 | 1.5283 | 1.1263 | 2.0677 |

[DRS] Unlike the probit analysis on an area basis, the difference between the genders is not statistically significant for the 180-min exposures.

## C3.0 ORDINAL LOGISTIC REGRESSION ANALYSIS OF MIOSIS VERSUS DOSAGE AND GROUP

The unreliability of the probit analysis in estimating the probit slope demonstrated the need for another approach. Ordinal logistic regression has been previously used with success on CW agent toxicity data (Sommerville (2004)) and was applied on the present dataset. Six different models were used, and calculations on both an area and diameter basis were performed. The following table summarizes the models investigated. The A-series of models were performed on data expressed on a pupil area basis, and the D-series used data expressed on a pupil diameter basis.

**Table C-1.** Terms Investigated in the Various Ordinal Logistic Regression Model Fits

| ID | Basis | Terms in Model |
|---|---|---|
| A1 | Area | LogC Time Sex Time*Sex |
| A2 | Area | LogC Time Sex |
| A3 | Area | LogC Time |
| A4 | Area | LogC LogT Sex LogT*Sex |
| A5 | Area | LogC LogT Sex |
| A6 | Area | LogC LogT |
| D1 | Diameter | LogC Time Sex Time*Sex |
| D2 | Diameter | LogC Time Sex |
| D3 | Diameter | LogC Time |
| D4 | Diameter | LogC LogT Sex LogT*Sex |
| D5 | Diameter | LogC LogT Sex |
| D6 | Diameter | LogC LogT |

The results from Models A1, A2, A3, D1, D2 and D3 were used to estimate the probit slope (concentration) for swine GB miosis exposure (see Section 3.3). Models A4, A5, A6, D4, D5 and D6 were used in order to calculate a toxic load exponent for miosis exposures. Error estimates for the probit slope and toxic load exponent were also obtained. A summary of the probit slope values and error estimates is provided in Table 4.

## C3.1 Miosis Score on Area Basis (A Series Models).

### C3.1.1 Ordinal Regression as Function of logC, Time and Sex (Model A1).

```
Link Function: Normit

Response Information

Variable  Value  Count
ScoreA    0          5
          1         14
          2         11
          3          7
          Total     37

Factor Information

Factor  Levels  Values
Time         3  10, 60, 180
Sex          2  F, M

Logistic Regression Table

Predictor        Coef   SE Coef      Z      P
Const(1)     -18.9655   4.06471  -4.67  0.000
Const(2)     -16.0572   3.45269  -4.65  0.000
Const(3)     -14.5661   3.33963  -4.36  0.000
logC         -24.6507   5.29980  -4.65  0.000
Time
  60         -17.2678   3.81733  -4.52  0.000
 180         -24.2180   5.22267  -4.64  0.000
Sex
 M           0.776908  0.777510   1.00  0.318
Time*Sex
  60*M       -1.15970   1.03900  -1.12  0.264
 180*M        2.37727   1.10412   2.15  0.031


Tests for terms with more than 1 degree of freedom

Term         Chi-Square  DF      P
Time            21.5883   2  0.000
Time*Sex         9.1614   2  0.010

Log-Likelihood = -28.605
Test that all slopes are zero: G = 40.013, DF = 6, P-Value = 0.000

Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      53.0832  81  0.993
Deviance     46.1199  81  0.999

Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant     441     90.2  Somers' D              0.81
Discordant      44      9.0  Goodman-Kruskal Gamma  0.82
Ties             4      0.8  Kendall's Tau-a        0.60
Total          489    100.0
```

## C3.1.2 Ordinal Regression as Function of logC, Time and Sex (Model A2).

```
Link Function: Normit

Response Information

Variable  Value  Count
ScoreA    0          5
          1         14
          2         11
          3          7
          Total     37


Factor Information

Factor  Levels  Values
Time         3  10, 60, 180
Sex          2  F, M

Logistic Regression Table

Predictor       Coef    SE Coef       Z      P
Const(1)    -13.7684    2.90404   -4.74  0.000
Const(2)    -11.5719    2.54826   -4.54  0.000
Const(3)    -10.3267    2.45900   -4.20  0.000
logC        -17.5397    3.84652   -4.56  0.000
Time
  60        -12.6395    2.84878   -4.44  0.000
 180        -16.2497    3.59976   -4.51  0.000
Sex
 M          0.805109   0.433166    1.86  0.063


Tests for terms with more than 1 degree of freedom

Term  Chi-Square  DF      P
Time     20.4124   2  0.000


Log-Likelihood = -34.071
Test that all slopes are zero: G = 29.081, DF = 4, P-Value = 0.000


Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       58.1028  83  0.983
Deviance      57.0526  83  0.987

Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs        Number  Percent  Summary Measures
Concordant      406     83.0  Somers' D              0.67
Discordant       79     16.2  Goodman-Kruskal Gamma  0.67
Ties              4      0.8  Kendall's Tau-a        0.49
Total           489    100.0
```

## C3.1.3 Ordinal Regression as Function of logC and Time (Model A3).

```
Link Function: Normit

Response Information

Variable  Value  Count
ScoreA    0          5
          1         14
          2         11
          3          7
          Total     37

Factor Information

Factor  Levels  Values
Time         3  10, 60, 180


Logistic Regression Table

Predictor      Coef  SE Coef       Z      P
Const(1)   -11.2450  2.36036   -4.76  0.000
Const(2)   -9.26135  2.08889   -4.43  0.000
Const(3)   -8.11153  2.00602   -4.04  0.000
logC       -14.5323  3.25124   -4.47  0.000
Time
  60       -10.4685  2.41918   -4.33  0.000
 180       -13.3931  3.02896   -4.42  0.000


Tests for terms with more than 1 degree of freedom

Term  Chi-Square  DF      P
Time     19.5799   2  0.000


Log-Likelihood = -35.856
Test that all slopes are zero: G = 25.512, DF = 3, P-Value = 0.000


Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      65.1834  75  0.784
Deviance     56.8018  75  0.942


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant     392     80.2  Somers' D              0.62
Discordant      91     18.6  Goodman-Kruskal Gamma  0.62
Ties             6      1.2  Kendall's Tau-a        0.45
Total          489    100.0
```

C3.1.4 Ordinal Regression as Function of logC, logT, Sex, logT*Sex (Model A4).

```
Link Function: Normit

Response Information

Variable  Value  Count
ScoreA    0          5
          1         14
          2         11
          3          7
          Total     37


Logistic Regression Table

Predictor       Coef    SE Coef       Z      P
Const(1)   -0.886272   0.662899   -1.34  0.181
Const(2)    0.550480   0.658678    0.84  0.403
Const(3)    1.63990    0.692402    2.37  0.018
logC       -7.44592    2.00445    -3.71  0.000
logT       -5.64394    1.55787    -3.62  0.000
Sex        -0.596146   0.631077   -0.94  0.345
logT*Sex    0.462720   0.364460    1.27  0.204


Log-Likelihood = -41.015
Test that all slopes are zero: G = 15.194, DF = 4, P-Value = 0.004


Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       86.8667  83  0.364
Deviance      70.9395  83  0.825


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant     371     75.9  Somers' D              0.53
Discordant     113     23.1  Goodman-Kruskal Gamma  0.53
Ties             5      1.0  Kendall's Tau-a        0.39
Total          489    100.0
```

[DRS] From variance-covariance matrix returned by MINITAB:

$var(k_C) = 4.01781$
$var(k_T) = 2.42696$
$covar(k_C, k_T) = 3.03978$

SE = sqrt (var)
$SE(k_C) = sqrt(4.01781) = 2.0$
$SE(k_T) = sqrt(2.42696) = 1.6$

toxic load exponent (n) = $k_C / k_T = (7.46) / (5.64) = 1.32$

Standard error (SE) for n (see Section C4.0)

$SE = (1.32)\ sqrt\ [\ (4.01781) / (7.4)^2 + (2.42696) / (5.6)^2 - (2)(3.03978) / (7.46) / (5.64)\ ] = 0.084$

C3.1.5 Ordinal Regression as Function of logC, logT and Sex (Model A5).

```
Link Function: Normit

Response Information

Variable  Value  Count
ScoreA    0          5
          1         14
          2         11
          3          7
          Total     37


Logistic Regression Table

Predictor       Coef    SE Coef      Z      P
Const(1)   -0.932754   0.660685  -1.41  0.158
Const(2)    0.485373   0.651034   0.75  0.456
Const(3)     1.54500   0.680729   2.27  0.023
logC        -6.96198    1.95185  -3.57  0.000
logT        -5.24514    1.51661  -3.46  0.001
Sex         0.171367   0.191509   0.89  0.371


Log-Likelihood = -41.803
Test that all slopes are zero: G = 13.618, DF = 3, P-Value = 0.003


Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       84.4449  84  0.466
Deviance      72.5150  84  0.810


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant     364     74.4  Somers' D              0.50
Discordant     118     24.1  Goodman-Kruskal Gamma  0.51
Ties             7      1.4  Kendall's Tau-a        0.37
Total          489    100.0
```

[DRS] From variance-covariance matrix returned by MINITAB:

$var(k_C) = 3.80973$
$var(k_T) = 2.30012$
$covar(k_C, k_T) = 2.87861$

SE = sqrt (var)
$SE(k_C) = sqrt(3.80973) = 2.0$
$SE(k_T) = sqrt(2.30012) = 1.5$

toxic load exponent (n) = $k_C / k_T = (6.96) / (5.25) = 1.33$

Standard error (SE) for n (see Section C4.0)

$SE = (1.33)\ sqrt\ [\ (3.80973) / (6.96)^2 + (2.30012) / (5.25)^2 - (2)(2.87861) / (6.96) / (5.25)\ ] = 0.090$

C3.1.6 Ordinal Regression as Function of logC and logT (Model A6).

```
Link Function: Normit

Response Information

Variable  Value  Count
ScoreA    0          5
          1         14
          2         11
          3          7
          Total     37


Logistic Regression Table

Predictor       Coef   SE Coef       Z      P
Const(1)   -0.983732  0.659000   -1.49  0.135
Const(2)    0.428577  0.644659    0.66  0.506
Const(3)     1.46117  0.673462    2.17  0.030
logC        -6.40232   1.83916   -3.48  0.000
logT        -4.80277   1.42771   -3.36  0.001


Log-Likelihood = -42.201
Test that all slopes are zero: G = 12.821, DF = 2, P-Value = 0.002


Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      74.0675  76  0.541
Deviance     69.4935  76  0.688


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant     360     73.6  Somers' D              0.49
Discordant     122     24.9  Goodman-Kruskal Gamma  0.49
Ties             7      1.4  Kendall's Tau-a        0.36
Total          489    100.0
```

[DRS] From variance-covariance matrix returned by MINITAB:

$var(k_C) = 3.38253$
$var(k_T) = 2.03835$
$covar(k_C, k_T) = 2.54425$

SE = sqrt (var)
$SE(k_C) = sqrt(3.38253) = 1.8$
$SE(k_T) = sqrt(2.03835) = 1.4$

toxic load exponent (n) = $k_C / k_T$ = (6.40) / (4.80) = 1.33

Standard error (SE) for n (see Section C4.0)

$SE = (1.33)\ sqrt\ [\ (3.38253) / (6.40)^2 + (2.03835) / (4.80)^2 - (2)(2.54425) / (6.40) / (4.80)\ ] = 0.090$

## C3.2 Miosis Score on Equivalent Diameter Basis.

### C3.2.1 Ordinal Regression as Function of logCT and Group (Model D1).

```
Link Function:  Normit

Response Information

Variable  Value       Count
ScoreD    0               9
          1              14
          2              14
          Total          37

Factor Information

Factor   Levels Values
Group         6 M10  M60  M180 F10  F60  F180

Logistic Regression Table

Predictor       Coef    SE Coef        Z      P
Const(1)       6.832      1.962     3.48  0.000
Const(2)       8.878      2.197     4.04  0.000
LogCT        -21.662      5.056    -4.28  0.000
Group
 M60          0.7493     0.8432     0.89  0.374
 M180          7.263      1.912     3.80  0.000
 F10          -1.2052    0.9562    -1.26  0.207
 F60           1.1039     0.8526     1.29  0.195
 F180          5.259      1.467     3.59  0.000

Tests for terms with more than 1 degree of freedom

Term      Chi-Square    DF      P
Group         15.963     5  0.007

Log-likelihood = -22.931
Test that all slopes are zero: G = 34.008, DF = 6, P-Value = 0.000

Goodness-of-Fit Tests

Method      Chi-Square    DF      P
Pearson         45.344    52  0.731
Deviance        34.772    52  0.968

Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs           Number  Percent     Summary Measures
Concordant         411    91.7%     Somers' D                0.85
Discordant          32     7.1%     Goodman-Kruskal Gamma    0.86
Ties                 5     1.1%     Kendall's Tau-a          0.57
Total              448   100.0%
```

## C3.2.2 Ordinal Regression as Function of logC, Time, and Sex (Model D2).

```
Link Function: Normit

Response Information

Variable  Value  Count
ScoreD    0          9
          1         14
          2         14
          Total     37


Factor Information

Factor  Levels  Values
Time         3  10, 60, 180
Sex          2  F, M


Logistic Regression Table

Predictor      Coef   SE Coef      Z      P
Const(1)   -13.1241   2.85178  -4.60  0.000
Const(2)   -11.3524   2.62196  -4.33  0.000
logC       -17.8052   3.96087  -4.50  0.000
Time
  60       -12.5281   2.87987  -4.35  0.000
 180       -16.6171   3.71618  -4.47  0.000
Sex
 M         0.667894  0.465694   1.43  0.152


Tests for terms with more than 1 degree of freedom

Term  Chi-Square  DF      P
Time     20.1768   2  0.000


Log-Likelihood = -25.284
Test that all slopes are zero: G = 29.302, DF = 4, P-Value = 0.000


Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      49.2797  54  0.657
Deviance     39.4786  54  0.931


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant     384     85.7  Somers' D              0.73
Discordant      58     12.9  Goodman-Kruskal Gamma  0.74
Ties             6      1.3  Kendall's Tau-a        0.49
Total          448    100.0
```

## C3.2.3 Ordinal Regression as Function of logC and Time (Model D3).

```
Link Function: Normit

Response Information

Variable  Value  Count
ScoreD    0          9
          1         14
          2         14
          Total     37


Factor Information

Factor  Levels  Values
Time         3  10, 60, 180


Logistic Regression Table

Predictor      Coef  SE Coef       Z      P
Const(1)   -11.5140  2.50578   -4.59  0.000
Const(2)   -9.81289  2.29710   -4.27  0.000
logC       -15.8505  3.56213   -4.45  0.000
Time
  60       -11.1075  2.57398   -4.32  0.000
 180       -14.7279  3.32460   -4.43  0.000


Tests for terms with more than 1 degree of freedom

Term  Chi-Square  DF      P
Time     19.7016   2  0.000


Log-Likelihood = -26.369
Test that all slopes are zero: G = 27.133, DF = 3, P-Value = 0.000


Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      31.5768  49  0.975
Deviance     32.2832  49  0.969


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant     380     84.8  Somers' D              0.72
Discordant      58     12.9  Goodman-Kruskal Gamma  0.74
Ties            10      2.2  Kendall's Tau-a        0.48
Total          448    100.0
```

C3.2.4 Ordinal Regression as Function of logC, logT Sex, and logT*Sex (Model D4).

```
Link Function: Normit

Response Information

Variable  Value  Count
ScoreD    0          9
          1         14
          2         14
          Total     37


Logistic Regression Table

Predictor         Coef   SE Coef       Z       P
Const(1)    -0.311849  0.709714   -0.44   0.660
Const(2)      1.07047  0.730183    1.47   0.143
logC         -8.69142   2.22881   -3.90   0.000
logT         -6.60843   1.72612   -3.83   0.000
Sex        -0.0785905  0.692723   -0.11   0.910
logT*Sex     0.132572  0.398028    0.33   0.739


Log-Likelihood = -31.161
Test that all slopes are zero: G = 17.550, DF = 4, P-Value = 0.002


Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      54.9106  54  0.440
Deviance     51.2307  54  0.582


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant     355     79.2  Somers' D              0.59
Discordant      89     19.9  Goodman-Kruskal Gamma  0.60
Ties             4      0.9  Kendall's Tau-a        0.40
Total          448    100.0
```

[DRS] From variance-covariance matrix returned by MINITAB:

$var(k_C) = 4.96759$
$var(k_T) = 2.97950$
$covar(k_C, k_T) = 3.74568$

SE = sqrt (var)
$SE(k_C) = sqrt(4.96759) = 2.2$
$SE(k_T) = sqrt(2.97950) = 1.7$

toxic load exponent (n) = $k_C / k_T$ = (8.691) / (6.608) = 1.32

Standard error (SE) for n (see Section C4.0)

$SE = (1.32)\ sqrt\ [\ (4.96759) / (8.69)^2 + (2.97950) / (6.61)^2 - (2)(3.74568) / (8.69) / (6.61)\ ] = 0.079$

## C3.2.5 Ordinal Regression as Function of logC, logT and Sex (Model D5).

```
Link Function: Normit

Response Information

Variable  Value  Count
ScoreD    0          9
          1         14
          2         14
          Total     37


Logistic Regression Table

Predictor       Coef    SE Coef       Z       P
Const(1)   -0.316619   0.709018   -0.45   0.655
Const(2)    1.06280    0.728024    1.46   0.144
logC       -8.58671    2.20351    -3.90   0.000
logT       -6.52649    1.71047    -3.82   0.000
Sex         0.144562   0.205764    0.70   0.482


Log-Likelihood = -31.214
Test that all slopes are zero: G = 17.443, DF = 3, P-Value = 0.001


Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       56.5454  55  0.417
Deviance      51.3374  55  0.615


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant     354     79.0  Somers' D              0.59
Discordant      89     19.9  Goodman-Kruskal Gamma  0.60
Ties             5      1.1  Kendall's Tau-a        0.40
Total          448    100.0
```

[DRS] From variance-covariance matrix returned by MINITAB:

var($k_C$) = 4.85547
var($k_T$ ) = 2.92572
covar($k_C$ , $k_T$ ) = 3.66818

SE = sqrt (var)
SE($k_C$) = sqrt(4.85547) = 2.2
SE($k_T$ ) = sqrt(2.92572) = 1.7

toxic load exponent (n) = $k_C$ / $k_T$ = (8.59) / (6.52) = 1.32

Standard error (SE) for n (see Section C4.0)

SE = (1.32) sqrt [ (4.85547) / $(8.59)^2$ + (2.92572) / $(6.52)^2$ – (2)(3.66818) / (8.59) / (6.52) ] = 0.080

## C3.2.6 Ordinal Regression as Function of logC and logT (Model D6).

```
Link Function:  Normit

Response Information

Variable  Value       Count
ScoreD    0               9
          1              14
          2              14
          Total          37

Logistic Regression Table

Predictor       Coef    SE Coef        Z      P
Const(1)     -0.3626     0.7058    -0.51  0.607
Const(2)      1.0067     0.7211     1.40  0.163
logC          -8.154      2.121    -3.84  0.000
logT          -6.184      1.647    -3.76  0.000

Log-likelihood = -31.459
Test that all slopes are zero: G = 16.952, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method      Chi-Square    DF      P
Pearson         42.009    50  0.782
Deviance        42.464    50  0.767


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs           Number  Percent     Summary Measures
Concordant         357    79.7%     Somers' D               0.61
Discordant          82    18.3%     Goodman-Kruskal Gamma   0.63
Ties                 9     2.0%     Kendall's Tau-a         0.41
Total              448   100.0%
```

[DRS] From variance-covariance matrix returned by MINITAB:

$var(k_C) = 4.49888$
$var(k_T) = 2.71109$
$covar(k_C, k_T) = 3.39190$

SE = sqrt (var)
$SE(k_C) = sqrt(4.49888) = 2.1$
$SE(k_T) = sqrt(2.71109) = 1.6$

toxic load exponent (n) = $k_C / k_T = (8.15) / (6.18) = 1.32$

Standard error (SE) for n (see Section C4.0)

$SE = (1.32)\ sqrt\ [\ (4.49888) / (8.15)^2 + (2.71109) / (6.18)^2 - (2)(3.39190) / (8.15) / (6.18)\ ] = 0.084$

## C4.0 CALCULATION OF CONFIDENCE LIMITS FOR TOXIC LOAD EXPONENT FROM RESULTS OF ORDINAL LOGISTIC REGRESSION ANALYSIS

MINITAB® in its ordinal logistic regression analysis routine does not automatically provide confidence limits for toxic load exponents. The user using other information provided by MINITAB® must calculate these limits. To calculate the limits, values from the fitted model coefficients and the variance-covariance matrix are used in conjunction with the following relation. Barry (1978) gives the standard error of a ratio, $(\alpha / \beta)$, which is based upon the propagation of error formula for a ratio:

$$\text{std err of } \left(\frac{\alpha}{\beta}\right) = \left(\frac{\alpha}{\beta}\right)\sqrt{\left(\frac{\text{var}(\alpha)}{\alpha^2}\right) + \left(\frac{\text{var}(\beta)}{\beta^2}\right) - (2)\left(\frac{\text{cov}(\alpha,\beta)}{\alpha\beta}\right)} \qquad \text{(C1)}$$

where var(α), var(β), and cov(α, β) are the variance of the quantities, α and β, and their covariance, respectively. The 95% confidence limits for the ratio will equal (α / β) ± (2)(std err). For the case of the toxic load ratio, the ratio of interest is $(k_C / k_T)$.

## C5.0 LITERATURE CITED


1. Barry, B.A. Errors in Practical Measurement Science, Engineering and Technology. John Wiley & Sons, Inc.: NY, 1978.

2. Sommerville, DR, *Relationship Between the Dose-Response Curves for Lethality and Severe Effects for Chemical Warfare Nerve Agents*; Proceedings of the 2003 Joint Service Scientific Conference on Chemical and Biological Defense Research (17-20 November 2003); ECBC-SP-XXX. US Army Edgewood Chemical Biological Center; Aberdeen Proving Ground, MD, 2004 (in publication), UNCLASSIFIED.

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